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Fishery Bulletin

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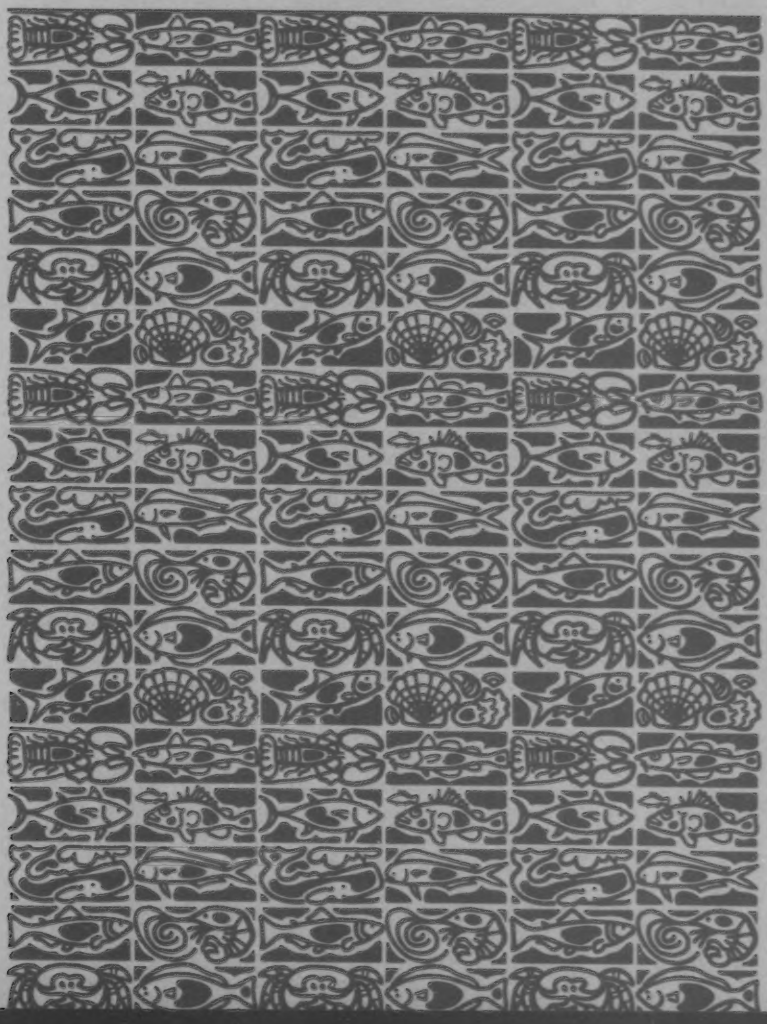

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Developmental instability as an indicator of environmental stress in the Pacific hake *(Merluccius productus)*

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Abstract.—Developmental Instability (DI) has been proposed as an inexpensive, quickly applied, and sensitive indicator of stress that can be utilized in early warning and in monitoring anthropogenic impacts on fish and other animals and plants. A problem arises, however, to the extent that natural stressors confound the effects of human-induced disturbances. Our objective in this work was to investigate whether a natural stressor, in the form of El Niño conditions, contributed to DI in the Pacific hake. Right-left (fluctuating) asymmetry of otolith length, width, growth rate, and weight, as well as right-left otolith shape differences, were used as measures of DI. Results show that indeed El Niño disrupts development, indicating stress. This outcome suggests that DI, as an early warning and monitoring tool for stress, must be used with caution.

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The viability of fish populations and the ecological communities of which they form a part is a matter of concern to fisheries managers. At present, awareness of ecological problems often occurs only when stocks have already begun to decline, when there is clear evidence of disease or morbidity, or when die-offs occur.

Much work has been done on methods to assess adverse environmental impacts on fish via physiological stress measures (Adams, 1990a). These methods can, in theory, be used to monitor problems and, possibly, act as early warning indicators. However, most of these indicators either measure short-term acute stress response (e.g., corticosteroid levels), or are expensive and laboratory-intensive. They are cost-effective only in evaluating already obvious signs that a problem exists. In economically depressed areas, including the developing nations, these methods are impractical. In addition, they generally lack ecological relevance (Adams, 1990b).

A possible alternative to these methods lies in morphological measures of developmental instability by which changes (induced by environmental or genetical stress) in the basic developmental strategy of organisms can be assessed. For example, chronic stress might aggravate right-left asymmetry in normally bilaterally symmetric structures (Soule, 1967; Valentine and Soule, 1973; Valentine et al, 1973) or increase the number of aberrations in circulus patterns on scales (Shackell and Doyle, 1990). Morphological measures are inexpensive to obtain and cause minimal or no damage to animals involved. They can be quickly and inexpensively applied not only in situations where concern has already arisen, but also as standard monitoring tools. Screening of fry might be valuable in assessing effectiveness of various hatchery practices or in evaluating smolt quality in Pacific salmon.

The efficacy of right-left, or "fluctuating" asymmetry (FA) as an indicator of both genetic and environmental stress has been well documented. Within populations, homozygosity is associated with increased FA

(Dobzhansky and Wallace, 1953; Lerner, 1954; Lewontin, 1956; Bader, 1965; Bruckner, 1976; Soule, 1979; Vrijenhoek and Lerman, 1982; Leary et al., 1983, 1984; Mitton and Grant, 1984). Increased homozygosity is one consequence of inbreeding (one form of genomic disruption). Within populations of outcrossing species, inbreeding is associated with lowered fitness (Lewontin, 1956; Charlesworth and Charlesworth, 1987). Bader (1965), Bailit et al. (1970), and Clarke (1992) have all found positive correlations among populations between inbreeding and FA.

The converse of inbreeding depression is outbreeding depression. Hybridization and subsequent introgression of sufficiently *different* populations can lead to the disruption of coadapted gene complexes and thus, in theory, promote both decreased fitness and developmental instability (Vrijenhoek and Lerman, 1982). Zakharov and Bakulina (1984) found that when individuals of similar populations of *Drosophila virilis* were crossed, no increases in FA over the parental types were observed. When more divergent strains were crossbred, FA was pronounced in the offspring. Outbreeding depression should be most apparent in interspecific hybrids. Leary et al. (1985) have found FA to be higher in laboratory hybrids of rainbow (*Oncorhynchus mykiss*) and cutthroat (*O. clarki*) trout than in either parental type.

Environmental disturbance, or deviation from the conditions to which organisms are adapted, leads to lowered fitness and developmental instability. Temperature stress causes increased FA in laboratory mice and rats (Beardmore, 1960; Siegel and Doyle, 1975, a and b; Siegel et al., 1977), in lizards (genus *Lacerta*, Zakharov 1982), and in chum salmon (*O. keta*, Beacham 1990). Audiogenic stress has the same effect on laboratory rats (Siegel and Smookler, 1973; Siegel and Doyle, 1975c). Valentine and Soule (1973) demonstrated that FA of laboratory grunion (*Leuresthes tenuis*) populations rose with concentrations of DDT. Clarke (1992) showed increased FA in pesticide-treated bush flies (*Musca vetustissima*) even at concentrations too low to produce statistically detectable changes in mortality. Zakharov and Rubin (1985) demonstrated the effects of contamination on FA in animals in the Baltic Sea. Thus FA may be an extremely sensitive indicator of stress.

Chronic stress indicators may be sensitive to naturally occurring events as well as anthropogenic disturbances. Zakharov et al. (1991), for example, found that intensified population density feedback led to increased FA during the decline phase of shrew population cycles. Data exist (Emlen, unpubl.) suggesting increased asymmetry occurs in the canine teeth of population-stressed northern fur seals. Clearly, if natural stressors are important contributors to developmental instability, then their effects must be considered when using DI as a tool for identifying or monitoring anthropogenic disturbances. In this paper we provide an example of FA responses to another natural stressor, the El Niño event of 1982–1983.

The Pacific hake (*Merluccius productus*) ranges along the Pacific coast of North America from Mexico to Alaska. Several genetic stocks can be distinguished. These include two spawning in Puget Sound, one from the Georges Straits, several from the fjords of Vancouver Island, and a so-called coastal population that ranges from San Francisco Bay to southern Baja California. Reproduction occurs from January to April, and young fish enter the fishery at about age three. Individual growth occurs largely between May and September.

Environmental conditions associated with the 1982–1983 El Niño may have caused significant dislocations of the coastal hake stock on the summer fishing grounds, since the entire population apparently moved northward (Francis and Hollowed, 1985). Length and weight information from the data set used by Francis and Hollowed and gathered by the National Marine Fisheries Service showed no significant differences between the 1980 and 1984 year classes for either sex. However, the 1980 El Niño year-class fish were slightly longer and lower in weight, and a Mann-Whitney test showed them to have significantly lower condition factor (weight/length^3) than the 1984 control year-class (respective means=0.06709, 0.07208; n=85, 63; P=0.0001). Both the dislocation in position and the diminished condition factor of population members suggest a drop in population viability and, thereby, implicate stress. Might this stress also have provoked developmental instability?

Methods

The development of otoliths, small calcareous structures used in maintaining balance in some fishes, is known to reflect growth rate and transition points in life histories (Wilson and Larkin, 1982; Volk et al., 1984; Alhossaini and Pitcher, 1988; Sogard, 1991). As such, they may be useful indicators of developmental instability. We do not suggest that otoliths fit the criteria given above for cost effectiveness and harmlessness to the organisms monitored. We use them here simply as a convenience (they were readily available and came with appropriate, attendant data on fish age, length, weight, and date of collection). The National Marine Fisheries Service, at its Alaska center in Seattle, maintains a collection of otoliths taken by scientific observers aboard commercial hake fishery boats. From this collection we obtained paired right and left

otoliths for 4-year-old hake caught in the first half of September for the years 1984 and 1988 (from the 1980 and 1984 year classes). These fish were the same from which the length and weight data, mentioned above, were taken. Individuals from the 1980 year class were in their second and third year of growth during El Niño; those from the 1984 year class were not present during the El Niño event. Time-sequential data is useful in the present context since the population was used as its own control, reducing or minimizing confounding factors of geography, genetic stock, etc.

Five otolith characters were used to assess instability: fluctuating asymmetry in weight, length, maximum width, growth rate, and right-left differences in shape (Fig. 1). For comparison of shape, one otolith was aligned with the reverse image of the other, and right-left shape differences examined. At least two means of alignment exist. Since otoliths show annual growth rings, it was possible to superimpose long axes drawn through the first growth ring. In the 1980 year class this ring was laid down just prior to the El Niño event and indicates the beginning of the period of stress. Measured differences along the outlines of the third ring, marking the end of El Niño, provide an appropriate measure of right-left differences in growth during the period. This approach was impractical.

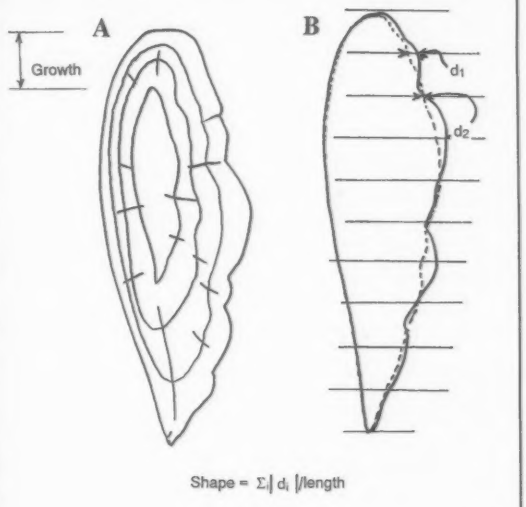

Figure 1

Hake *(Merluccius productus)* otoliths: **(A)** An otolith showing growth lines and the measure used to assess growth between ages 1 and 4; **(B)** Diagram of superimposed right and left otoliths showing how shape difference was characterized.

While distances between the rings could be reasonably approximated with the aid of an optical micrometer, our equipment was inadequate to permit video or photo reproduction of clear ring patterns. Accurate axes could not be drawn. Minute angular changes in axis definition generated large differences in the shape measure.

Because of these difficulties, we used an alternative approach. Equally magnified images of the two otoliths were projected onto paper and their outlines traced. One tracing was then turned over and placed atop the other. Each otolith possesses clearly marked "top" and "bottom" extreme points (Fig. 1), so alignment could be accomplished by superimposing the axes drawn through these two points. This procedure led to an almost perfect fit of the two otoliths along their straighter side and to clear pattern differences along their opposite scalloped side (Fig. 1). Nine equally spaced lines were drawn perpendicular to this axis, and, along each such line, the absolute distance between the scalloped margins of the two overlain outlines was measured. Right-left differences among the otoliths were taken as the sum of these absolute distances, normalized by dividing by the axis length. Information lost by neglecting similar differences along the straight side was negligible owing to the almost perfect fits along that margin. Growth rate was measured by the maximum distance, parallel to the straight side, between the first and the fourth otolith growth ring at the broader end of the otolith (Fig. 1).

Four-year-old fish caught in September, near the end of the growth season, may have displayed some variance in size owing to differences in date of hatching. This variance is not likely to be large because little growth occurs between earliest and latest hatching in January and April. Nevertheless, to correct for possible associations between asymmetry and early growth, we used a normalized index, $|L-R|/(L+R)$, with L and R designating left and right measures. Because collections for each of the three years were made at very nearly the same time, biases arising from possible influences of growth stage on asymmetry were minimized. The measure of right-left difference in shape is not a measure of FA in the strict sense (Palmer and Strobeck, 1986). However, an increase in the measure would indicate an increased deviation from normal developmental homeostasis. Thus it is a valid indicator of developmental instability.

Tests for fluctuating asymmetry depend on an absence of directional asymmetry (skew), anitsymmetry (bimodality or platykurtosis) and, according to one school of thought (Palmer and Strobeck, 1986), a normal distribution for (L–R)/(L+R). The Shapiro-Wilk statistic (Zar, 1984, p. 95) was used to test for normality. Because of the controversy surrounding the validity of

FA tests for traits not normally distributed, further analyses were carried out twice, once with and once without such traits. A second order ANOVA was applied to test for influences of sex and year class on growth rate. Finally, fish were catergorized as fast or slow growers, according to whether growth rate fell above or below the median for the year in question, and a three-way MANOVA was performed to test for effects of growth rate, sex, and year on FA and right-left otolith shape differences.

Results

For both the 1980 and 1984 year classes, we found that the distributions of weight, width, and length did not differ significantly from normality (Table 1). These variables can, therefore, be used in analysis of FA. Growth rate was not normally distributed. The two-way ANOVA indicated no influence of sex (F=1.42; df=1, 141; P=0.234) and no significant interaction effect of sex X year (F=0.36; df=1,141; P=0.550) on growth. The main effect of year, however, was strong (F=14.20; df=1,141; P=0.0002). This last result, along with the possibility that growth rate might influence asymmetry of other characters, provided the rationale for incorporating growth rate into the three-way MANOVA, as noted above. Results were qualitatively the same—no differences with respect to which comparison deviated significantly—whether growth rate was or was not included in the MANOVA, and so only one result set, that including this variable, is presented in this paper (Table 2). There was a highly significant difference in developmental instability, but only between the years. Univariate analysis showed that FA was greater for the El Niño fish with respect to otolith weight and shape, but not width or length (Tables 3 and 4).

To examine the possibility that the between-year differences were not due to El Niño effects, we examined otoliths of 4-year-old fish from another year class (1977) unaffected by El Niño events. As with the above two groups, fish sampling took place during the first two weeks of September. These otoliths were analyzed for right-left asymmetry in weight and shape. Results of a Multiple Analysis of Variance for all three year classes, with fixed effects of year and sex, are given in Tables 5 and 6. The results, in conjunction with Tukey tests for differences among years (Table 7) show clear increases in right-left differences in both shape and weight for the 1980 El Niño year over 1984 and, in shape, over 1977 as well. Differences between the two years not affected by El Niño (1977 and 1984) were not statistically significant.

Table 1

Tests for normality of (L–R)/(L+R) for various traits used in developmental instability. (L indicates measure on the left, R the corresponding measure on the right otolith).

Width of otolith (n = 147)
Mean = 0.002
Skew = 0.184
Kurtosis = 1.333
Shapiro-Wilk W = 0.990
P = 0.9456

Weight of otolith (n = 135)
Mean = 0.003
Skew = 0.242
Kurtosis = 0.683
Shapiro-Wilk W = 0.994
P = 0.9965

Length of otolith (n = 135)
Mean = 0.001
Skew = 0.049
Kurtosis = 2.030
Shapiro-Wilk W = 0.975
P = 0.1981

Growth rate of otolith (1980 year class; n = 79)
Mean = –0.001
Skew = –1.567
Kurtosis = 6.124
Shapiro-Wilk W = 0.981
P = 0.0001

Growth rate of otolith (1984 year class; n = 69)
Mean = 0.002
Skew = –0.281
Kurtosis = 0.248
Shapiro-Wilk W = 0.981
P = 0.7044

Discussion

The observation that only two of the five right-left asymmetry measures responded to stress at a statistically detectable level raises the question of consistency. If developmental instability is to be a useful indicator of stress, we need to know what measures will be relatively sensitive to stress and which will not. Highly

Table 2

Multiple analysis of variance for various traits used in developmental instability analysis. Fixed effects are year, sex and growth rate. Variables are right-left differences in width, weight, length, growth rate, and shape.

Effects	Wilks Lambda	F	df	P
Year	0.8029	5.596	5, 114	0.0001
Sex	0.9509	1.176	5, 114	0.3253
Growth	0.9532	1.119	5, 114	0.3546
Year × sex	0.9407	1.438	5, 114	0.2159
Year × growth	0.9230	1.901	5, 114	0.0995
Sex × growth	0.9612	0.961	5, 114	0.4704
Year × sex × growth	0.9936	0.146	5, 114	0.9807

Table 3

Univariate breakdown of multivariate analysis of variance for various traits used in developmental instability analysis.

Fixed effect	df	SS	*F*	*P*
Variable				
Weight				
Growth rate	1	0.00001	0.26	0.608
Sex	1	0.00009	2.28	0.133
Year	1	0.00036	9.07	0.003
Growth × sex	1	0.00010	2.63	0.107
Growth × year	1	0.00001	0.35	0.554
Sex × year	1	0.00008	2.09	0.151
3-Way	1	0.00002	0.49	0.487
Error	118	0.00469		
Width				
Growth rate	1	0.00000	0.00	0.951
Sex	1	0.00003	0.88	0.350
Year	1	0.00008	2.08	0.152
Growth × sex	1	0.00001	0.32	0.574
Growth × year	1	0.00030	2.54	0.114
Sex × year	1	0.00004	1.15	0.286
3-Way	1	0.00000	0.01	0.930
Error	118	0.00456		
Growth rate				
Growth rate	1	0.00134	4.11	0.045
Sex	1	0.00058	1.77	0.186
Year	1	0.00073	2.24	0.137
Growth × sex	1	0.00000	0.00	0.998
Growth × year	1	0.00068	2.10	0.150
Sex × year	1	0.00067	2.06	0.154
3-Way	1	0.00007	0.22	0.639
Error	118	0.03837		
Shape				
Growth rate	1	0.00065	0.76	0.385
Sex	1	0.00015	0.17	0.679
Year	1	0.01856	21.79	0.000
Growth × sex	1	0.00220	2.58	0.111
Growth × year	1	0.00076	0.89	0.347
Sex × year	1	0.00292	3.43	0.066
3-Way	1	0.00006	0.07	0.753
Error	118	0.10049		
Length				
Growth rate	1	0.00002	0.60	0.440
Sex	1	0.00001	0.31	0.581
Year	1	0.00000	0.00	0.986
Growth × sex	1	0.00002	0.62	0.433
Growth × year	1	0.00009	2.66	0.106
Sex × year	1	0.00001	0.39	0.534
3-Way	1	0.00000	0.01	0.933
Error	118	0.00409		

Table 4

Means of developmental instability indices of Pacific hake *(Merluccius productus)* otoliths for 1980 and 1984 year classes.

	1980 (El Niño Group)				1984 (Non El Niño Group)			
Variable	Males	(*n*)	Females	(*n*)	Males	(*n*)	Females	(*n*)
Weight	0.0083	(36)	0.0117	(36)	0.0065	(24)	0.0065	(30)
Shape	0.0831	(36)	0.0950	(36)	0.0679	(24)	0.0603	(30)

Table 5

Multiple analysis of variance over one El Niño year (1980) and two Non-El Niño years (1977, 1984). Fixed effects are year and sex. Variables are weight and shape.

Effects	Wilks Lambda	*F*	df	*P*
Year	0.7606	11.876	4, 324	0.000
Sex	0.9930	0.574	2, 162	0.564
Year × sex	0.9577	1.770	4, 324	0.135

Table 6

Univariate breakdown of multiple analysis of variance for two traits used in developmental instability analysis over three years (1977, 1980, 1984).

Sum of squares		df	*F*	*P*
Variable = weight				
Sex	0.00002	1	0.63	0.430
Year	0.00038	2	5.01	0.008
Sex × year	0.00017	2	2.18	0.116
Error	0.00621	163		
Variable = shape				
Sex	0.00062	1	0.82	0.366
Year	0.03576	2	23.65	0.000
Sex × year	0.00285	2	1.89	0.155
Error	0.12322	163		

Table 7

Tukey test for differences among years (1977, 1980, 1984) in two measures of developmental instability. El Niño year class is 1980; Non El Niño year classes are 1977, 1984. NS = not significantly different.

Year class	Difference between means	(Sample sizes)	*P*
Shape			
1980–1984	0.0250	(77, 59)	<0.05
1980–1977	0.0337	(77, 25)	<0.05
1977–1984	–0.0087	(25, 59)	NS
Weight			
1980–1984	0.0033	(79, 55)	<0.05
1980–1977	0.0024	(79, 38)	NS
1977–1980	0.0009	(38, 55)	NS

Table 8
Means of asymmetry in growth rate for Pacific hake *(Merluccius productus)* in an El Niño and a non-El Niño year.

	Year	
	El Niño (1980)	Non El Niño (1984)
Growth rate		
Slow	0.0142	0.0132
Fast	0.0251	0.0157

canalized traits are unlikely to be sensitive indicators of stress. High phenotypic variance can generally be considered an indication of low canalization, and Zakharov (1989) has shown that, at least for *Lacerta* lizards, FA rises with phenotypic variability (see also Soule, 1967).

A related observation is that traits directly and strongly affecting fitness, i.e., under intense stabilizing selection and, therefore, with low heritability, should be poor candidates for detecting stress (Soule and Cuzin-Roudy, 1982). Similarly, structures whose development is affected by use may not be appropriate for FA analysis. Locomotion, for example, might discourage asymmetric growth of limb size. Long-bone length in laboratory rats shows no FA response to heat, cold, behavioral, or audiogenic stress, while long-bone density, not a size characteristic, does (Doyle et al., 1977).

In choosing measures of developmental instability, it may be useful to consider structural detail. With respect to otoliths, weight, length, width, and growth rate represent growth along a single dimension. These metrics, then, are a simple summation of one or more growth processes. Shape, on the other hand, reflects growth processes whose parts are separably measurable. Thus, shape does not compound and thereby obscure information. We might, accordingly, expect to find differences in the shapes of corresponding right and left structures to be more sensitive indicators of stress than simple metric differences, a prediction consistent with the hake otolith results presented here.

Do natural environmental stressors, as well as man-caused disturbances, result in developmental instability? The results reported here suggest an affirmative answer, namely that while DI might be usefully applied in management, it must be used with caution. Use of fin asymmetries or scale circulus aberrations in comparing hatchery stocks for smolt quality, for example, should involve only a single year class and should be given decreasing consideration as stocks diverge genetically or geographically. Suspected problems arising from water contamination in Puget Sound might be investigated or monitored by examining FA, but only so long as the control population comes also from Puget Sound.

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Analysis of fishery resources: potential risk from sewage sludge dumping at the deepwater dumpsite off New Jersey

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Abstract.—Analytical and statistical procedures were applied to bottom trawl survey data in tests of hypotheses about potential effects of sewage sludge dumping at a 106-mile dumpsite (106-MDS) off New Jersey on fishery resources assessed on the continental shelf and upper slope. Sludge dumping, even in deep ocean waters, was not discounted as one of several ecological and environmental perturbations influencing these resources measured as temporal, spatial, and seasonal differences in abundance. Species abundances of silver and red hakes (*Merluccius bilinearis* and *Urophycis chuss*), summer flounder (*Paralichthys dentatus*), goosefish (*Lophius americanus*), and black sea bass (*Centropristis striata*) declined significantly over temporal and spatial scales during the disposal of contaminant-laden sewage sludge at the deepwater 106-MDS. There was also a decline in the array of all aggregated species, but to a lesser degree. Results of these analyses of assessment data are considered in relation to effects of ocean dumping in shallow waters at the southern California sewage outfalls and in the New York Bight apex, and in relation to increased contamination of the ecosystem around the 106-MDS. Further, large-scale coordination of environmental research surveys with fishery resource assessments would allow tests of more specific hypotheses and allow a more definitive interpretation of offshore resource population data as presented here.

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Sewage sludge and a variety of other wastes have been disposed of in coastal waters of the New York Bight for over 50 years (Squires, 1983). Offshore deepwater waste disposal of sewage sludge, however, is a relatively new activity of the last 20 years. With legally mandated closure of the coastal water 12-mile dump site (12-MDS) in the New York Bight apex (Ocean Dumping Ban Act of 1988), a deepwater dumpsite, 106 miles off New Jersey (106-MDS), was selected as a temporary alternative site for disposal of sewage sludge from the New York and New Jersey metropolitan area (O'Connor, 1983; O'Connor et al., 1983, 1985; Pearce et al., 1983; Norton, 1989; NOAA[1,2]; Battelle[3]).

The 106-MDS had been used previously for disposal of industrial wastes as well as sewage sludge (O'Connor, 1983; Bisagni[4]; Anderson[5]). Phased relocation of New York-New Jersey sewage disposal began in March 1986, and by December 1987 all sewage sludge disposed at sea was being barged to the 106-MDS site. At the 106-MDS, an average of 6.0 million metric tons (t) wet weight sewage sludge (range 1.2–9.9 million t) was dumped between 1986 and 1992. This mean is similar to an average of 5.8 million t of sewage sludge (range 4.0–8.3 t) dumped at the 12-MDS from 1973 to 1987. New Jersey ceased disposal at 106-MDS in March 1991. New York City phased out 20% of its ocean dumping in December 1991, and the remainder by the end of June 1992.

The fishing industry, citizens groups, state governments, and federal agencies all voiced concerns about potential effects of heavy sewage sludge dumping at the 106-MDS on the surrounding marine environment and fisheries. Dumping of sewage sludge and industrial waste in

[1]NOAA. 1975. May 1974 baseline investigation of deepwater dumpsite 106. NOAA Dumpsite Evaluation Report 75-1. U.S. Dep. Commer., Natl. Oceanic Atmospheric Admin., Natl. Ocean Survey, Rockville, MD, 388 p.

[2]NOAA. 1977. Baseline report of environmental conditions in deepwater dumpsite 106. NOAA Dumpsite Evaluation Report 77-1. U.S. Dep. Commer., NOAA, Natl. Ocean Survey, Rockville, MD, 798 p.

[3]Battelle. 1990. 106-miles deepwater municipal sludge site monitoring, research and surveillance plan. Battelle Memorial Institute, Duxbury, MA, 90 p.

[4]Bisagni, J. J. 1977. A summary of the input of industrial waste chemicals at deepwater dumpsite 106 during 1974 and 1975. NOAA Dumpsite Evaluation Report 77-1, U.S. Dep. Commer., NOAA, Natl. Ocean Survey, Rockville, MD, 487–497 p.

[5]Anderson, P. W. 1983. Current status—ocean dumping in the New York Bight. Paper presented on 6 April 1983 in Atlantic City, NJ at the 15th National Conference and Exhibition on Municipal and Industrial Sludge Utilization and Disposal, 6 p.

the comparatively pristine deep-water ocean environment has been considered to have an adverse effect on fishery resource abundance and composition (Carlise, 1969; Dart and Jenkins, 1981; Mearns, 1981; Russo, 1982; Cross et al., 1985; Spies, 1984; Farrington et al., 1982; Capuzzo and Kester, 1987, a and b; O'Connor et al., 1985, 1987, a and b; Werme[6,7]; SCCWRP[8]; Zdanowicz et al.[9]; NMFS[10,11]; Studholme et al.[12]). A contaminant distribution model presented by O'Connor et al. (1985) (see also Reed et al., 1985) indicated that New York and New Jersey sewage sludge has the immediate effect of increasing concentrations of polychlorinated biphenyls (PCB's), Zn, Pb, Cr and Cu. In addition, the potential area of influence (**PAI**) of waste disposal at the 106-MDS extends over a wide region (Fig. 1) off the Middle Atlantic outer continental shelf and upper slope (Bisagni, 1983; O'Connor et al., 1985; Gentile et al., 1989; Warsh[13], Bisagni[14], Ingham[15]).

A change in fish species population abundance and composition is known to have occurred near shallow-water sewage sludge disposal outfalls in southern California (Mearns 1981; Sherwood[16]). For example, a bothid flounder (Pacific sanddab, *Cithaichthys sordidus*) was replaced by a pleuronetcid (Dover sole, *Microstomus pacificus*). Increased growth rates and high prevalence of fin erosion of Dover sole, however, appear to have resulted from exposure to sediments contaminated with chemical wastes from the outfalls. Liver anomalies were noted as well and seem to have been associated with exposure to sludge-related contaminants (Mearns, 1981). Spies (1984) reported that distribution of bottom-feeding fishes (e.g., Dover sole) is probably affected by degraded benthic habitats and abundance of benthic (e.g., polychaete, *Capitella capitata*) and pelagic prey around these outfalls in the southern California Bight. Some chemical contaminants associated with the sewage accumulated at higher trophic levels in predatory fishes, e.g., Pacific sanddab and boccaccio, *Sebastes paucispinis*. Benthic-pelagic coupling was evident in the accumulation of chlorinated hydrocarbons in Dover sole and Pacific sanddab.

Cessation of sewage sludge dumping on the biomass of the most frequently occurring fish species around the coastal waters near 12-MDS from 1986 to 1989 was examined by Studholme et al.[12]. There were no significant changes, although American lobster (*Homarus americanus*) biomass increased (Pikanowski, 1992; Wilk et al.,[17]). Possibly this 39-month-long study period was insufficient to detect a recovery of any finfish species alterations resulting from decades of sewage dumping at the old 12-MDS. Interestingly, the high prevalence of fin-rot disease of winter flounder (*Pleuronectes americanus*) around the 12-MDS reported earlier (Ziskowski and Murchelano, 1975; Murchelano and Ziskowski, 1976) declined significantly after cessation of the dumping (O'Connor et al.[18]; Pacheco and Rugg[19]). It was also shown earlier that Atlantic mack-

[6]Werme, C., R. Shokes, W. Steinhauer, S. McDowell, P. Debrule, P. Hamilton, and P. Boehm. 1988a. Evaluation and recommendations for bioaccumulation studies for the 106-mile deepwater municipal sludge site monitoring program. Battelle Memorial Institute, Duxbury, MA, 36 p.

[7]Werme, C., K. M. Jop, S. Y. Freitas and P. Boehm. 1988b. Implementation plan for the 106-mile deepwater municipal sludge site monitoring. Battelle Memorial Institute, Duxbury, MA, 51 p.

[8]SCCWRP (Southern California Coastal Water Research Project). 1989. Recovery of Santa Monica Bay after termination of sludge discharge. Southern California Coastal Water Research Project. Annual Report 1988–1989, 46–53 p.

[9]Zdanowicz, V. S., M. C. Ingham, and S. Leftwich. 1990. Monitoring the effects of sewage sludge disposal at the 106-mile dumpsite using mid-water fish as sentinels of contaminant metal uptake: a feasibility study. U.S. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Northeast Fish. Sci. Cent., Woods Hole, MA. Ref. Doc. 90-02, 6 p.

[10]NMFS. 1992a. Interim report on monitoring the biological effects of sludge dumping at the 106-mile dumpsite. U.S. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Northeast Fish. Sci. Cent., Woods Hole, MA 02543, 128 p

[11]NMFS. 1992b. Second annual report on monitoring the biological effects of sludge dumping at the 106-mile dumpsite. U.S. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Northeast Fish. Sci. Cent., Woods Hole, MA 02543, 157 p

[12]Studholme, A. L., J. O'Reilly, and M. C. Ingham (eds.). 1993. Effects of the cessation of dumping at the 12-mile site. U.S. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Northeast Fish. Sci. Cent., Sandy Hook Lab., Highlands, NJ 07732. (In review.)

[13]Warsh, C. E. 1975. Physical oceanography hist. rical data for Deepwater Dumpsite 106. NOAA Dumpsite Evaluation Report 75-1. U.S. Dep. Commer., NOAA, Natl. Ocean Survey, Rockville, MD, 105–140 p.

[14]Bisagni, J. J. 1976. Passage of anticyclonic Gulf Stream eddies through Deepwater Dumpsite 106 during 1974 and 1975. NOAA Dumpsite Evaluation Report 76-1. U.S. Dep. Commer., NOAA, Natl. Ocean Survey, Rockville, MD, 39 p.

[15]Ingham, M. C. 1977. The general physical oceanography of deepwater dumpsite 106. NOAA Dumpsite Evaluation Report, 77-1. U.S. Dep. Commer., NOAA, Natl. Ocean Survey, Rockville, MD, 29–54 p.

[16]Sherwood, M. J. 1978. The fin erosion syndrome. Southern California Coastal Water Research Project. Annual Report 1978, 203–221 p.

[17]Wilk, S. J., R. A. Pikanowski, A. L. Pacheco, D. G. McMillan, and L. L. Stehlik. 1993. Response of fish and megainvertebrates of the New York Bight apex to the abatement of sewage sludge dumping: an overview. *In* A. L. Studholme, J. O'Reilly and M. C. Ingham (eds.), Effects of the cessation of dumping at the 12-mile site. U.S. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Northeast Fish. Sci. Cent., Woods Hole, MA, 14 p. (In review.)

[18]O'Connor, J. S., J. J. Ziskowski, and R. A. Murchelano. 1987. Index of pollutant-induced fish and shellfish disease. NOAA Spec. Rep. Ocean Assessment Div. U.S. Dep. Commer., NOAA, Natl. Ocean Survey, Rockville, MD, 29 p.

[19]Pacheco, A. L., and J. Rugg. 1993. Disease incidence of inner New York Bight winter flounder collected during the 12-mile dumpsite study, 1986–1989. *In* A. L. Studholme, J. O'Reilly, and M. C. Ingham (eds.), Effects of the cessation of dumping at the 12-mile site. U.S. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Northeast Fish. Sci. Cent., Sandy Hook Lab., Highlands, NJ, 14 p. (In review.)

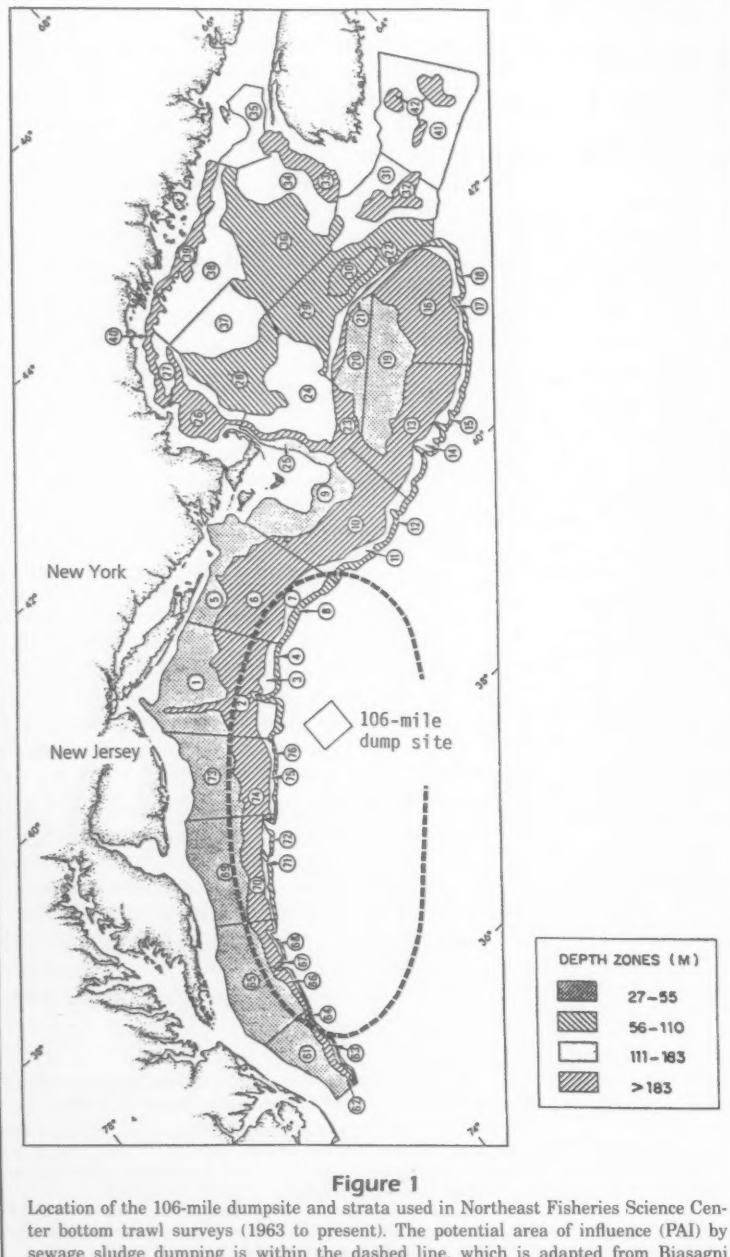

Figure 1

Location of the 106-mile dumpsite and strata used in Northeast Fisheries Science Center bottom trawl surveys (1963 to present). The potential area of influence (PAI) by sewage sludge dumping is within the dashed line, which is adapted from Biasagni (1983).

erel (*Scomber scombrus*) embryos developing in surface waters about the 12-MDS (1974–1978) had greater mortality and gross malformation, more mitotic abnormality and mitotic inhibition than those in less contaminated bight areas, and this was linked to site contamination (Chang and Longwell, 1984; Longwell,1988; Longwell et al., 1992).

Unlike the 12-MDS in the New York Bight apex and the sewage outfalls in southern California, the 106-MDS is situated in a physically dynamic zone characterized by periodic shifts and overturns in water masses. The toxic water is rapidly diluted and dispersed. This may diminish the impact of dumped waste and associated chemical contaminants, but it also makes determination of any adverse effects on the resource more difficult to study than those associated with sewage sludge dumping in shallow-waters. Still, the economic value of the nearby fisheries warrants some effort at directly measuring potential adverse impacts of the 1986–92 sludge dumping on the fishery resource in the vicinity of the 106-MDS.

The study reported here is an attempt to determine if any change in fishery resource abundance on the adjacent continental shelf and slope could be detected after sewage sludge disposal commenced at the 106-MDS in March 1986. Although ocean dumping is now banned, this period of sludge dumping in the deep ocean provides an interesting case study for consideration of effects of ocean pollution in general on economically important fishery resources. This analysis is of potential interest in respect to any future reconsideration of ocean dumping. There are no prior population level studies on abundance changes in marine fishes associated with the sewage sludge dumping in deep ocean waters.

The general null hypothesis that was tested is that no change in resource species abundance and composition was coincident with sewage dumping at the deepwater 106-MDS during the period 1986–90. Assessments were made of temporal, spatial, and seasonal differences in 11 individual finfish

species, and of all these species combined. The general hypothesis was subdivided into three more specific hypotheses, each of which was likewise tested: 1) there are no *temporal* differences in species abundance between the period prior to sewage sludge dumping at the 106-MDS and the period after dumping resumed; 2) there are no *spatial* differences in species abundance between the area north of the 106-MDS and the area south of the dump site, the latter more likely to be influenced by the sewage sludge that supposedly moved through the dump site toward the west-southwest shallow outer shelf (PAI; Fig. 1); and 3) there are no *seasonal* differences for individual and all species for the north and south areas within the pre- and post-dumping periods.

Methods

Data from NOAA's Northeast Fisheries Science Center (NEFSC) bottom trawl surveys were used for analysis. From 1963 to the present, NEFSC has conducted bottom trawl surveys on the Northwest Atlantic Shelf and upper slope, from the Scotian Shoals to Cape Hatteras including the areas adjacent to the 106-MDS (Fig. 1). The entire survey area has been stratified by depth down to 365 m, and surveys have been based on a stratified random design that provides statistically valid samples for estimating indices of species population abundance. All fishes and invertebrates are sorted, counted, and weighed by species to the nearest 0.1 kg. Large catches are subsampled by weight and volume and expanded to estimate the entire catch. Detailed information pertinent to the rationale, descriptions of trawl gears and sampling schemes, as well as history of the NEFSC bottom trawl survey are provided by Grosslein (1974) and more recently Azarovitz (1981). Grosslein et al. (1979) summarized research, assessment, and management of the northwest Atlantic ecosystem using data from NEFSC bottom trawl surveys.

Data from the spring 1982 through spring 1986 NEFSC surveys were evaluated as the pre-dumping period, and data from autumn 1986 through spring of 1990 as the post-dumping period. Eleven economically important species were selected for analysis: silver hake (*Merluccius bilinearis*); red hake (*Urophycis chuss*); summer flounder (*Paralichthys dentatus*); goosefish (*Lophius americanus*); black sea bass (*Centropristes striatus*); scup (*Stenotomus chrysops*); butterfish (*Peprilus triacantus*); longfin squid (*Loligo pealei*); American lobster (*Homarus americanus*); sea scallop (*Placopecten magellanicus*); and spiny dogfish (*Squalus acanthias*). Species were selected on the basis of their commercial values and landings, and the known availability of data for the entire 106-MDS study area (Chang, 1990). In addition, all species, a category comprising the total catch for each cruise, was also used.

The multivariate rank sum test (Puri and Sen, 1971) was employed for temporal, spatial, and seasonal differences in the population abundance analyses by using the catch per unit of effort (CPUE) as a measure of population abundance: 1) the *average* CPUE values for each bottom trawl survey stratum for all cruises occurring in the pre-dumping period cruises (spring 1982–spring 1986), and for all cruises occurring in the post-dumping period cruises (autumn 1986–spring 1990), and 2) the average CPUE data from the area defined as south of the 106-MDS, including strata 61–76 and data from the area defined north of the site including strata 1–12 (Fig. 1). Test statistics for CPUE were calculated and compared with critical values from the chi-square table to determine the following:

1. there are no temporal differences in CPUE of pre- and post-dumping periods for spring and autumn cruises collectively in the regions north and south of the 106-MDS (cf. Table 1),
2. there are no spatial differences in CPUE north and south of the 106-MDS for pre- and post-dumping period cruises collectively in spring and autumn seasons (cf. Table 2),
3. there are no seasonal differences in CPUE of spring and autumn surveys for pre- and post-dumping period cruises collectively in the regions of north and south of the 106-MDS (cf. Table 3).

Test statistics were also computed and compared with the critical values for testing CPUE for spatial differences for individual species and for all species taken on each cruise in the area north and south of the 106-MDS. Test statistics and abundance indices of individual species in tabulated form are not included among tables given here.

The values of test statistics for temporal, spatial, and seasonal differences are readily comparable (Tables 1–3). When examining values of the test statistics for temporal differences, secondary differences for species, regions, and seasons were also assessed. Test statistics were similarly examined for spatial and seasonal differences among species, regions, and seasons.

Changes in significance or non-significance of the test statistics between pre- and post-dumping period surveys may be interpreted as indicative of temporal differences in CPUE. Significant changes of test statistics between regions north and south of the 106-MDS indicate spatial differences. Changes between spring and autumn surveys indicate seasonal differences. Significant differences in CPUE in time, space, and season may thus be interpreted as some shift in species abundance. *Negative* differences are taken here as a response of the population to some adverse

Table 1

Results of the rank sum test for **temporal** differences in species catch per unit of effort (CPUE) between pre- and post-dumping periods using spring and autumn cruises collectively in north and south regions.

	Pre-dumping period vs. post-dumping period					
	Spring Cruises			Autumn Cruises		
Species name	CPUE (weight)	CPUE (number)	No. of species	CPUE (weight)	CPUE (number)	No. of species
North						
Silver hake	0.098	4.709*		2.064	0.621	
Red hake	0.596	0.075		10.087*	3.320	
Summer flounder	8.553*	2.349		1.409	1.817	
Goosefish	2.290	0.001		15.520*	1.192	
Black sea bass	0.423	0.140		0.482	2.964	
Scup	0.127	1.035		7.656*	2.910	
Butterfish	7.288*	11.810*		1.361	0.161	
Longfin squid	0.225	0.039		4.443*	0.001	
American lobster	3.170	0.168		1.184	0.639	
Sea scallop	3.626	5.285*		1.199	1.736	
Dogfish	17.166*	10.993*		0.003	0.094	
All species[1]	10.077*	4.094*	2.503	0.671	0.051	3.278
South						
Silver hake	1.472	0.040		0.966	6.984*	
Red hake	6.391*	4.576*		0.707	0.700	
Summer flounder	6.645*	10.785*		1.219	1.440	
Goosefish	0.934	0.118		3.664	0.033	
Black sea bass	5.207*	6.474*		0.386	0.240	
Scup	1.184	0.077		0.943	0.087	
Butterfish	0.147	0.050		8.443*	12.691*	
Longfin squid	1.801	1.395		2.548	0.327	
American lobster	0.247	2.792		1.713	1.539	
Sea scallop	16.057*	12.614*		0.973	1.600	
Dogfish	0.242	0.043		0.362	1.297	
All species[1]	14.641*	21.212*	1.834	9.099*	2.377	8.473*

[1] "All species" includes the eleven listed and all other species.
* Significant difference with the critical values at 95% level in the chi-square table.

biological or environmental perturbation, to fishing pressure or to some combination of these factors. *Positive* differences are taken as increased species abundance in response to favorable conditions. Nonetheless, significant differences of this sort alone do not establish a direct causal association between change in population abundance and sewage sludge dumping, or alternately between fishing pressure, natural environmental perturbations, or any of combination of these. Any such inference must come from further interpretation of the data in respect to what is known about natural ecology of the subject species, fishing pressure on these, and any known sensitivities to sewage sludge.

Abundance indices for the 11 species individually and for all species combined for pre- and post-dumping period surveys (Tables 4–7, pgs. 601–604) were computed by using the groundfish survey analysis program (Kramer[20]). Basic assumptions and methodology for estimation of abundance indices based on NEFSC bottom trawl survey data are detailed by Pennington and Brown (1981). Trends in indices and variabilities in time and space may also be interpreted as suggesting some general shift in species abundance attributable to sludge dumping, even though there may be no direct proof of an association between population abundance change and sludge disposal.

Results

Temporal differences

Test statistics based on species CPUE, and species abundance indices, including estimates for both spring and autumn cruises, are summarized in Tables 1 and 4–7. Combining the information from these two sources reinforces the inferences of significant change in species abundance over time.

[20] Kramer, W. P. 1985. Groundfish survey analysis program (SURVAN version 5.2), program report. Prepared for U.S. Dep. of Commer., NOAA,. Natl. Mar. Fish. Serv., Northeast Fish. Cent., Input Output Computer Service Inc., Waltham, MA. 137 p.

Table 2

Results of the rank sum test for **spatial** differences in species catch per unit of effort (CPUE) between north and south regions using pre- and post-dumping period cruises collectively in spring and autumn seasons.

	North region vs. south region					
	Pre-dumping cruises			Post-dumping cruises		
Species name	CPUE (weight)	CPUE (number)	No. of species	CPUE (weight)	CPUE (number)	No. of species
Spring						
Silver hake	13.402*	28.189*		17.765*	35.719*	
Red hake	3.654	6.591*		15.014*	22.364*	
Summer flounder	5.833*	5.161*		2.356	0.624	
Goosefish	0.228	0.469		0.467	0.023	
Black sea bass	0.259	0.727		1.956	2.217	
Scup	2.483	1.295		0.511	8.128*	
Butterfish	12.030*	10.791*		0.496	0.154	
Longfin squid	1.201	4.823*		0.004	7.375*	
American lobster	0.160	1.747		2.235	6.298*	
Sea scallop	0.001	0.351		5.503*	4.505*	
Dogfish	16.084*	3.279		0.115	1.561	
All species[1]	1.078	0.943	11.532*	0.113	6.313*	8.808*
Autumn						
Silver hake	40.064*	8.555*		41.868*	24.898*	
Red hake	33.139*	17.622*		11.277*	7.116*	
Summer flounder	11.877*	1.987		12.920*	1.263	
Goosefish	46.468*	4.564*		24.837*	0.819	
Black sea bass	6.133*	2.938		14.058*	0.304	
Scup	0.532	2.283		0.018	0.729	
Butterfish	5.400*	0.062		16.728*	10.153*	
Longfin squid	10.717*	10.898*		5.531*	12.555*	
American lobster	0.178	1.747		0.140	4.371*	
Sea scallop	0.635	1.157		0.288	0.639	
Dogfish	10.896*	3.279		29.296*	18.483*	
All species[1]	24.920*	9.559*	5.992*	50.827*	21.397*	43.765*

[1] "All species" includes the eleven listed and all other species.
*Significant difference with the critical values at 95% level in the chi-square table.

Analyses of red hake data are used here as an example of a detailed interpretation of temporal differences in species CPUE. (Black sea bass could be equally well used because of its similar pattern of CPUE indices.) Species test statistics for red hake revealed significant temporal differences in CPUE indices both by weight (biomass) and number of individuals in the south, but not in the north region (Table 1). Biomass indices declined from pre-dumping spring cruises to post-dumping spring cruises (Table 4). Numerical abundance indices also declined from pre-dumping spring cruises to post-dumping spring cruises (Table 5). Red hake autumn data were also analyzed, but the species test statistics were not significant (Table 1). Biomass indices (Table 6) and number indices (Table 7) declined from the pre-dumping to the post-dumping period. These negative temporal differences for red hake are interpreted as an indication of significant reduction in abundance over time.

CPUE test statistics for several other species also suggest significant temporal differences between pre- and post-dumping periods. The number of significant differences was greater for spring cruises than for autumn cruises (Table 1). Biomass indices were relatively low for both spring and autumn cruises in both north and south regions generally, with the exception of high values for dogfish (Tables 4 and 6). Species number indices were mixed (Tables 5 and 7).

Test statistics for summer flounder and sea scallop CPUE by weight and number for the spring cruises were significantly different in the southern region. In the northern region, only summer flounder biomass and scallop number indices were significantly different. Indices for summer flounder and sea scallop for autumn cruises, however, failed to be significant in either region (Table 1). Summer flounder abundance indices of both biomass and number indicate a decline from the pre-dumping period to the post-dumping period, while both indices for the sea scallop increased (Tables 5 and 7). This suggests a decline (negative differences) over time for summer flounder, and an enhancement (positive differences) for sea scallop.

Table 3

Results of the rank sum test for testing **seasonal** differences in species catch per unit of effort (CPUE) between spring and Autumn seasons using pre- and post-dumping period cruises collectively in north and south regions.

	Spring season vs. autumn season					
	Pre-dumping cruises			Post-dumping cruises		
Species name	CPUE (weight)	CPUE (number)	No. of species	CPUE (weight)	CPUE (number)	No. of species
North						
Silver hake	1.444	1.473		8.382*	2.653	
Red hake	8.745*	4.845*		0.057	0.291	
Summer flounder	5.024*	0.471		8.835*	1.378	
Goosefish	0.098	4.898*		3.337	0.681	
Black sea bass	6.519*	2.850		8.597*	0.009	
Scup	0.078	2.757		2.498	6.681*	
Butterfish	1.123	0.945		6.645*	14.920*	
Longfin squid	9.991*	21.686*		0.262	14.070*	
American lobster	0.988	0.033		4.137*	0.683	
Sea scallop	4.774*	4.137*		1.490	0.806	
Dogfish	11.294*	4.527*		20.414*	16.008*	
All species[1]	15.317*	41.133*	1.944	57.442*	22.629*	0.265
South						
Silver hake	35.795*	2.322		27.903*	2.594	
Red hake	6.125*	0.291		0.707	0.108	
Summer flounder	0.035	0.150		0.002	0.045	
Goosefish	28.811*	0.509		16.162*	0.131	
Black sea bass	3.476	0.506		10.751*	4.645*	
Scup	6.210*	3.409		0.019	0.085	
Butterfish	4.236*	16.741*		0.292	0.742	
Longfin squid	3.229	0.342		0.762	3.974*	
American lobster	0.095	5.804*		0.013	0.689	
Sea scallop	6.425*	4.257*		0.289	0.206	
Dogfish	16.927*	8.076*		53.442*	37.763*	
All species[1]	51.240*	12.546*	0.919	140.185*	7.022*	24.325*

[1] "All species" includes the eleven listed and all other species.
* Significant difference with the critical values at 95% level in the chi-square table.

Biomass and number test statistics for butterfish in spring cruises were significantly different between pre- and post-dumping periods in the northern region, but not significantly different in the southern region. Conversely, those statistics from autumn cruises were not significant in the northern region, but significant in the southern region. Test statistics for spiny dogfish showed similar patterns (Table 1). Species abundance indices for butterfish and spiny dogfish fluctuated, but generated much higher values for the test statistics than did those of other species (Tables 4–7). Accordingly, interpretation of the butterfish and spiny dogfish statistics suggests a positive difference in these two species over time.

In spring cruises, test statistics in CPUE both by weight and number for goosefish, scup, longfin squid, American lobster, and silver hake were not significantly different for pre- and post-dumping periods for either the north or south region, with exception of silver hake number indices in the north region. For autumn cruises, however, there were significant differences between pre- and post-dumping periods for goosefish, scup, and longfin squid (Table 1). Abundance indices for goosefish declined from the pre- to the post-dumping period for both cruises and regions. Longfin squid indices declined from the pre to the post period in spring cruises, but increased in autumn cruises. All scup indices declined but increased in autumn cruises within the south region. American lobster indices showed little change between pre- and post-dumping periods. Silver hake indices increased in spring cruises but declined in autumn cruises from the pre to post period (Tables 4–7). The lower abundance indices of these species may signify that the populations responded negatively to adverse differences.

In spring cruises, CPUE statistics for all species, both by biomass and number, were significantly different between pre- and post-dumping periods in both regions. This occurred though test statistics for some individual species were significantly different and

Table 4

Species abundance indices (e.g., mean and SSE [standard error]) based on **weight** of stratified catch per tow and variabilities (CV in %) using pre- and post-dumping spring cruises collectively in north and south regions.

Species name	Pre-dumping spring cruises			Post-dumping spring cruises		
	Abundance indices			Abundance indices		
	Mean	SSE	CV (%)	Mean	SSE	CV (%)
North						
Silver hake	1.529	0.62509	41	1.217	0.31258	26
Red hake	1.549	0.78584	51	0.863	0.23815	28
Summer flounder	0.401	0.09030	23	0.211	0.05036	24
Goosefish	1.109	0.28732	26	0.656	0.14389	22
Black sea bass	0.104	0.05443	52	0.025	0.01060	42
Scup	0.510	0.23137	45	0.192	0.09896	52
Butterfish	1.820	1.03770	57	0.383	0.13748	36
Longfin squid	1.009	0.27199	27	2.038	0.55665	27
American lobster	0.440	0.19855	45	0.350	0.09429	27
Sea scallop	0.194	0.09369	48	0.877	0.38851	44
Dogfish	51.186	14.36400	28	115.671	36.00800	31
All spp.[1]	183.027	23.42700	13	259.398	38.04700	15
South						
Silver hake	0.426	0.14559	34	0.389	0.10256	26
Red hake	0.671	0.31264	47	0.147	0.06884	45
Summer flounder	0.413	0.10370	25	0.260	0.06533	25
Goosefish	0.938	0.33040	35	0.367	0.17910	49
Black sea bass	0.238	0.08226	20	0.429	0.22193	52
Scup	0.635	0.46357	73	1.293	0.61669	48
Butterfish	0.340	0.15347	45	0.682	0.36380	53
Longfin squid	0.811	0.20697	26	2.382	0.86509	36
American lobster	0.082	0.02381	29	0.151	0.07966	53
Sea scallop	0.092	0.03275	36	1.245	0.73640	59
Dogfish	92.764	36.88800	40	74.456	15.59700	21
All spp.[1]	226.566	53.61400	24	181.878	18.19100	10

[1] "All species" includes the eleven listed and all other species.

others not. In the autumn cruises, biomass statistics were statistically significant between pre- and post-dumping period cruises, but for the southern region only (Table 1). These results substantiate the negative temporal differences. They suggest a reduction over time for all species abundance and biomass in both regions of the study area.

Abundance indices for all species in spring cruises decreased from the pre- to the post-dumping periods but increased in autumn cruises. Variabilities for all species were consistently lower (9–24%) than those for individual species (18–88%) (Tables 4–7). This suggests that fluctuations in total biomass in the study area from 1982 to 1990 for all species were lower as a whole and masked fluctuations in individual species. One may infer that the impacts of fishing, ecological perturbations, or natural factors acting on all species as a whole, therefore, were relatively low in the spring. Although impact on the abundance indices for all species was less than on some individual species, there were no significant differences in the number of species between pre- and post-dumping periods in either region.

Spatial differences

To assess spatial differences for species CPUE between north and south regions, the same approach was used as for temporal differences. Test statistics for spatial differences are summarized in Table 2 and Tables 4–7. These are based on species CPUE, species abundance indices, and their variability estimates from collective cruises in pre- and post-dumping periods.

More species test statistics for autumn cruises were significantly different between the north and south

Table 5

Species abundance indices (e.g., mean and SSE [standard error]) based on **number** of stratified catch per tow and variabilities (CV in %) using pre- and post-dumping spring cruises collectively in north and south regions.

Species name	Pre-dumping spring cruises			Post-dumping spring cruises		
	Abundance indices			Abundance indices		
	Mean	SSE	CV (%)	Mean	SSE	CV (%)
North						
Silver hake	7.143	1.66440	23	9.255	2.13410	23
Red hake	6.491	2.35950	36	5.298	1.52160	29
Summer flounder	0.591	0.12461	21	0.350	0.07453	21
Goosefish	0.410	0.08432	21	0.285	0.05213	18
Black sea bass	0.410	0.23043	56	0.101	0.05330	58
Scup	3.027	1.76110	58	0.574	0.31650	55
Butterfish	28.105	13.54800	48	9.375	4.93800	53
Longfin squid	17.936	5.27820	29	37.478	15.55800	42
American lobster	0.995	0.46534	47	0.897	0.27397	31
Sea scallop	3.214	1.77760	55	35.384	23.24100	66
Dogfish	49.555	16.10700	33	79.592	22.47900	28
All spp.[1]	511.574	48.78400	10	554.673	51.95500	9
South						
Silver hake	2.431	0.57454	24	4.874	1.80180	37
Red hake	2.311	0.79196	34	0.641	0.21119	33
Summer flounder	1.016	0.31625	31	0.760	0.18100	24
Goosefish	0.314	0.07206	23	0.147	0.04088	28
Black sea bass	0.816	0.26722	33	2.007	0.71847	37
Scup	21.419	18.81900	88	17.119	8.51020	50
Butterfish	11.695	6.16760	53	51.810	44.22500	85
Longfin squid	50.430	21.27200	42	119.842	57.41000	48
American lobster	0.133	0.03483	26	0.137	0.04451	32
Sea scallop	1.845	0.62564	34	25.703	17.49900	68
Dogfish	45.598	16.41000	36	39.621	7.73790	20
All spp.[1]	450.046	47.42500	11	675.431	70.50500	10

[1] "All species" includes the eleven listed and all other species.

regions than those for spring cruises (Table 2). Several patterns are evident in significant test statistics for the autumn cruises. Biomass CPUE for eight species showed highly significant differences for both pre- and post-dumping periods. These species were silver hake, red hake, summer flounder, goosefish, black sea bass, butterfish, longfin squid, spiny dogfish, and all species. Fewer significant differences were observed in the number of CPUE. No distinct pattern was obvious for spring cruises. Only silver and red hake CPUE showed significant test statistics for both periods. Scup, American lobster, and sea scallop CPUE showed generally lower values that were not significantly different. There was a significant difference between north and south in number of species for autumn cruises in both the pre-dumping and post-dumping periods (Table 2).

Interpretation of these significant spatial differences requires special caution. In 1982, the first year for which data were used in this study, populations were not of comparable size in the north and south regions. One must consider how populations of unequal size could have accommodated differing environmental perturbations and fishing pressure in the north and south regions. Silver hake, red hake, butterfish, and spiny dogfish indices were highest. Goosefish and American lobster indices were lower overall, but higher in the north than in the south, while scup were higher only in the south. Longfin squid indices were consistent with its migratory pattern (concentrated in the south in spring and in the north in autumn) (Tables 4–7). Test statistics of all eight species were significantly different between north and south (Table 2).

Test statistics were tabulated for species CPUE between north and south, and for species abundance indices, including all species in the north and south regions for individual cruises, but are not presented here. This information supplemented the findings from collective cruises (Tables 2, 4–7). Indices from individual cruises

Table 6

Species abundance indices (e.g., mean and SSE [standard error]) based on **weight** of stratified catch per tow and variabilities (CV in %) using pre- and post-dumping autumn cruises collectively in north and south regions.

Species name	Pre-dumping spring cruises			Post-dumping spring cruises		
	Abundance indices			Abundance indices		
	Mean	SSE	CV (%)	Mean	SSE	CV (%)
North						
Silver hake	1.573	0.31258	23	0.997	0.30246	31
Red hake	2.707	0.69497	26	0.380	0.09313	25
Summer flounder	0.328	0.08221	25	0.135	0.06060	45
Goosefish	1.539	0.34043	22	0.350	0.10007	29
Black sea bass	0.010	0.00598	60	0.005	0.00363	73
Scup	0.905	0.40331	45	0.203	0.07233	36
Butterfish	9.356	3.27910	35	7.924	2.74850	35
Longfin squid	7.771	2.77170	36	4.266	0.94622	22
American lobster	0.539	0.10552	20	0.630	0.11589	18
Sea scallop	0.284	0.10900	38	1.810	1.07450	59
Dogfish	53.835	32.34200	60	19.980	13.61500	68
All spp.[1]	129.958	21.28800	16	76.576	7.16770	9
South						
Silver hake	0.178	0.05296	30	0.060	0.01787	30
Red hake	0.225	0.11908	53	0.052	0.02049	39
Summer flounder	0.439	0.16250	37	0.072	0.02495	34
Goosefish	0.111	0.03029	27	0.032	0.01329	42
Black sea bass	0.082	0.04975	61	0.060	0.02440	41
Scup	1.779	1.05160	59	4.660	3.96680	85
Butterfish	2.605	1.04430	40	2.803	1.43660	51
Longfin squid	2.716	0.64098	24	1.433	0.35703	25
American lobster	0.125	0.02937	24	0.069	0.02283	33
Sea scallop	0.278	0.14392	52	1.126	0.50005	44
Dogfish	0.006	0.00351	59	0.022	0.00672	31
All spp.[1]	65.832	11.32200	17	44.661	9.18910	21

[1] "All species" includes the eleven listed and all other species.

provided insights into how individual fish and shellfish populations responded in time and space. The variabilities were relatively consistent in both regions, despite confounding biotic and abiotic factors. Fluctuations of total biomass for eleven individual species, and for all species as a whole, were relatively stable in both north and south regions during the period 1982–90.

Seasonal differences

Seasonal differences between spring and autumn were determined by the same method used to evaluate temporal and spatial differences. Test statistics for seasonal differences based on species CPUE and species abundance indices with their variability estimates were drawn from collective cruises of the pre- and post-dumping periods. They are summarized in Tables 3 and 4–7.

Species test statistics for the pre-dumping cruises showed more significant differences between spring and autumn seasons than did post-dumping cruises. Weight test statistics provided more significant differences than those based on number. Spiny dogfish CPUE revealed significant test statistics for all categories. Test statistics for black sea bass, based on both biomass and number, showed significant differences between seasons for the post-dumping cruises in the southern region, but not for the pre-dumping cruises in the same region. Similarly, silver hake, butterfish, and American lobster indices in the northern region were significantly different for the post-dumping cruises, but not significantly different for pre-dumping cruises (Table 3). Some species weight abundance indices declined from the pre-dumping to post-dumping periods for both spring and autumn seasons (Tables 4 and 7). These included silver and red hakes, summer flounder, goosefish, and black sea bass. Number of species for the post-dumping cruises in the southern region was also significantly different (Table 3).

Table 7

Species abundance indices (e.g., mean and SSE [standard error]) based on **number** of stratified catch per tow and variabilities (CV in %) using pre- and post-dumping autumn cruises collectively in north and south regions.

Species name	Pre-dumping spring cruises			Post-dumping spring cruises		
	Abundance indices			Abundance indices		
	Mean	SSE	CV (%)	Mean	SSE	CV (%)
North						
Silver hake	32.869	9.57110	29	16.073	4.51730	28
Red hake	13.974	3.72120	27	2.968	0.72162	24
Summer flounder	0.268	0.07093	26	0.135	0.05503	41
Goosefish	0.658	0.13661	21	0.334	0.07429	22
Black sea bass	0.530	0.24114	46	0.137	0.05997	44
Scup	9.652	3.80610	39	7.770	5.12530	66
Butterfish	191.717	60.39400	32	241.977	87.52200	36
Longfin squid	307.679	141.09000	46	297.160	92.28000	31
American lobster	1.245	0.24816	20	1.573	0.30140	19
Sea scallop	4.165	1.58340	30	39.042	25.79400	66
Dogfish	32.848	18.35200	56	13.260	8.71090	66
All spp.[1]	1481.352	162.12000	11	1365.058	154.03000	11
South						
Silver hake	8.632	2.41370	28	3.340	1.03060	31
Red hake	1.436	0.65891	46	0.482	0.19790	41
Summer flounder	1.136	0.40306	22	0.200	0.06627	33
Goosefish	0.252	0.05769	23	0.128	0.03517	27
Black sea bass	1.003	0.62278	62	0.823	0.36718	45
Scup	40.626	24.08400	59	149.944	132.31000	88
Butterfish	85.958	32.22600	37	84.561	47.39100	56
Longfin squid	107.343	33.92100	32	74.365	38.98300	52
American lobster	0.152	0.03600	24	0.142	0.04323	30
Sea scallop	5.443	3.00540	55	22.849	9.84010	43
Dogfish	0.039	0.02346	60	0.131	0.03651	28
All spp.[1]	842.080	117.52000	14	815.743	133.00000	16

[1] "All species" includes the eleven listed and all other species.

Discussion

Analyses of fisheries abundance data, based on catch per unit of effort (CPUE) of NOAA's Northeast Fisheries Science Center (NEFSC) bottom trawl surveys, indicate that abundances of silver hake, red hake, summer flounder, goosefish, and black sea bass declined about the 106-mile dumpsite (106-MDS) from spring of 1982 through spring of 1990. These declines are concurrent with the temporary dumping there of sewage sludge from the New York and New Jersey metropolitan area. Changes in total abundance and biomass for all species combined occurred to a lesser degree because of increased abundance of spiny dogfish, skates (*Raja* spp.) and some pelagic species (e.g., Atlantic mackerel) (NMFS 1991, 1992). There were statistically significant, temporal differences in average abundance values for some individual species and for all species based on spring and autumn bottom trawl surveys within the north and south dumpsite areas. It is particularly worth noting that much of the abundance decline occurred in the south area of the dumpsite. The south region is most likely to be influenced by sewage sludge introduced in waters passing toward the west-southwest shallow outer shelf through the dumpsite in the potential area of influence (**PAI**) of the sewage sludge (Fig. 1; Bisagni, 1983; O'Connor et al., 1985; Gentile et al., 1989; Ingham[15]; Warsh[13]; Bisagni[4]). There were also statistically significant spatial differences in average abundance of many individual species and all species sampled on the spring and autumn surveys within the pre- and post-dumping period at the 106-mile dumpsite. There were seasonal differences, as well, in average values for sev-

eral individual species and all species for north and south areas at the site, and within the pre and post sludge dumping periods.

The hypothesis that fishery resource abundance in the vicinity of the 106-MDS and adjacent outer continental shelf waters was not affected by sewage sludge dumping there from 1986 to 1990 is thereby rejected. Also rejected are the three secondary hypotheses related to the primary one. The likelihood remains that waste disposal, even in deep-ocean waters, has measurable adverse impacts on the resource abundance, at least in the area of direct influence of the toxic waste.

Analysis of survey data alone, however, does not establish cause and effect. It is necessary to consider the likelihood that events neither directly nor indirectly related to sewage sludge dumping at the 106-MDS could have led to the measured decline in abundance and shift in species composition within the study period. Also, it is necessary to consider how an abundance shift could have occurred through sludge dumping within this period of time.

Fishing is foremost among factors affecting fishery resource abundance and species composition unrelated to any effect of ocean disposal of toxic waste. Impacts of fishing pressure on the species sampled at the offshore dumpsite and analyzed in this study are poorly known. There are also other poorly understood natural factors affecting species population abundance around the 106-MDS in the PAI and elsewhere that could have influenced abundance and species composition at this site over the period of the study reported here. These include shifts in spawning time and area, size of predator stocks, decline in size of the spawning biomass, and increase in early life mortality (Gross, 1976; Hempel, 1978; Mayer, 1982; Cross et al., 1985; Tiews, 1985). The dramatic reductions in stock size affected by powerful modern fishing techniques, however, through sheer reduction in stock size, may influence all these factors so that their fluctuation is no longer entirely natural. These same natural factors are likewise potentially affected by ocean dumping of toxic waste as evidenced in the environmental literature (Carlise, 1969; Gross, 1976; Mearns, 1981; Mayer, 1982; Spies, 1984; Wolf and O'Connor, 1988; Champ and Park, 1989; Hood et al., 1989; Baumgartner and Duedall, 1990; Longwell et al. 1992; Longwell[21]; Young and Mearns[22]; NMFS[10,11]; Studholme et al.[12]). Changes in water mass patterns and global warming are possibly the only phenomena not potentially influenced by ocean dumping. Given the complexity of natural and anthropogenic forces acting on populations, existing data sets from fishery ecology are simply not sufficiently synoptic or complete enough to be useful in sorting out direct sludge dumping effects from natural or pseudo-natural processes driving population fluctuation.

Oceanographic dynamics around the 106-MDS make it unlikely that any change in harvestable fishery resources or catch composition could be the result of direct mortality attributable to the sewage since relocated sludge dumping began there in 1986. There is some evidence from embryo studies of planktonic eggs collected in the wake of sludge and acid waste disposal at 106-MDS that direct kills of floating fish eggs can occur, but this dumpsite is not a significant spawning grounds for resource species (Longwell[21]).

Changes in fishery resource abundance at 106-MDS could, however, have been affected by a change in fish behavior in response to dumped material as well as to natural environmental perturbations (Olla et al., 1980). Migration to other areas to escape sludge disposal and its aftermath or to search for other feeding grounds is likely to alter the time and place of spawning and subsequent early-life survival.

Reduced reproductive success and recruitment resulting from increased baseline contaminant body burdens of spawners is another mechanism whereby the population changes measured in the study presented here could have resulted from sludge dumping (Cross and Hose, 1988; Longwell et al., 1992). Offshore contamination of fish has the same potential of affecting subsequent spawning and recruitment as does contamination in nearshore waters and on the spawning grounds. Shifts in species composition could have come about if predator or competitor, or both, species were more tolerant of the sludge than were the resource species. In addition, poor quality eggs could have resulted from inadequate maternal nutrition if there was much direct prey mortality or pollution-impaired reproduction of prey organisms in the wake of the sludge. Contaminant burdens of the reproductive tissues or ripe eggs of the species analyzed in this study are unknown. Extrapolation and formal treatment of existing data on muscle and liver tissues of these or on the reproductive tissues of other species are probably not worthwhile. Contaminant burdens of mature winter flounder eggs alone (Calabrese et al.[23]) suggest that

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safety margins for adverse reproductive effects of several contaminants (Susani, 1986; Sorenson, 1991) on fish species at the 106-MDS could easily have been exceeded. Only moderate concentrations of chlorinated hydrocarbons have been shown to be associated with detrimental reproductive effects (Susani, 1986; Westernhagen et al., 1989).

An interdisciplinary effort was made to determine changes in contaminant burdens of fish at the 106-MDS once sludge dumping began (NMFS[10,11]). In 1991, concentrations of metals were relatively low in the epibenthic megafauna species of deepwater fishes, such as blue hake (*Antimora rostrata*), rattails (*Coryphaenoides carapinus* and *C. armatus*), halosaur (*Halosauropsis macrochir*) and commercially valuable slope-dwelling tilefish (*Lopholatilus chamaeleonticeps*), shrimp (*Nematocarcinus ensifer* and *Glyphocrangon sculpta*), and American lobster (*Homarus americanus*). This was generally true for American lobster, with the exception of elevated liver Cd, which could reflect its trans-shelf migration and possible exposure to coastal pollution. Finfishes, however, generally contained relatively high concentrations of chlorinated pesticides and total PCB's in their livers (NMFS[10,11]). Significantly elevated levels of several metals (Ag, Cu,and Cr) were found in midwater myctophids (e.g., *Benthosema glaciale*, *Lobianchia dofleini*, *Ceratoscopelus maderensis*, and *Hygophum hygomii*) at stations southwest of the 106-MDS in the principal area of influence of the sludge.

Plankton (primarily copepods) provide a pathway for entry of potentially toxic chemical contaminants from the sludge into the ocean food-web, because it constitutes the main prey of smaller midwater fishes. Concentrations of metals in plankton samples taken in 1991 were comparable to, or higher than, those observed in fishes collected in 1989. The geographic distributions of metal concentrations in 1991 fishes and plankton suggest that the elevated levels of metals found in certain samples are probably attributable to dumping activity at the 106-MDS. Boehm (1983), however, postulated that offshore, southerly transport of organic contaminants along the New Jersey shore and down-valley transport from the New York Bight apex caused increased metal contaminants. Organic contaminants (PCB's, polynuclear aromatic hydrocarbons (PAH's) and pesticides) were present in lower concentrations in zooplankton than in midwater fishes (NMFS[10,11]).

The highest concentration patterns of PAH's, PCB's, and Ag in the sediment surface layer (0–0.5 cm) tended to follow the distribution patterns of spores of *Clostridium perfringens* (a bacterial indicator of sewage) in sediments collected from depths of 100 to 2800 m in the vicinity of the 106-MDS. *Clostridium perfringens* counts declined gradually to the southwest. Surface sediments were not detectably contaminated with other trace metals. Organic contaminant concentrations in sub-surface strata (0.5–3.5 cm) were higher than in the sediment or deeper layers. Artifacts in sediment cores in the 106-MDS tend to confirm that chemical concentration is related to dumping. All data imply that material dumped at the 106-MDS reached the seabed in the area southwest of the site in the PAI. Data imply that the chemicals associated with the sludge entered the food-web around the dumpsite in the area believed to have been under principal influence of the sludge (NMFS[10,11]). This is the area accounting for the most of the decline in fish species measured in this study.

Unless absorbed by particles or precipitated by other constituents in the water column, slowly sinking wastes must take a long time to reach the bottom because of the great water depth. This increases the likelihood of significant ingestion of waste and associated contaminants by mid-water column prey species of resource fish. Deepwater food-web dynamics have been explored in the 106-MDS by Van Dover et al. (1992) by using the natural stable isotopes of organic carbon nitrogen and sulphur in sewage sludge to trace sewage-oriented organic matter. Organic matter from the sludge was found to reach the deep-sea floor and enter the benthic food-web through consumption by surface-deposit feeders, including a sea urchin (*Echinus affinus*) and a sea cucumber (*Benthodytes sanquinolenta*). Other surface-deposit feeders, including infaunal benthic species such as polychaetes and molluscs, probably also ingest this organic matter contributing to sludge impacts in the open ocean via food-chain dynamics.

There are strong associations of benthic macrofauna with habitat types and sediment contaminants in the continental shelf of the New York Bight (Chang et al., 1992). Species most common in the contaminated area around 12-MDS were mainly polychaetes (e.g., *Tharyx acutus*, *Nephtys incisa*, *Pherusa affinis*, and *Capitella* spp.), as well as a nemertean (*Cerebratulus lacteus*), an anemone (*Ceriantheopsis americanus*), a phoronid (*Phoronis architecta*) and the nut clam *Nucula proxima*. Another group of species was consistently associated with minimally contaminated sediments and appeared to represent a basic natural benthic macrofaunal assemblage for the typical sandy habitat of the Bight shelf. This group included the sand dollar (*Echinarachnius parma*) and several species of amphipods (e.g., *Byblis serrata*, *Corophium crassicorne*, and *Ampelisca agassizi*), as well as polychaetes (e.g., *Goniadella gracilis* and *Exogone hebes*). The roles of these contaminant-sensitive and insensitive species within

certain food-web dynamics for commercially important fisheries resources in the New York Bight are becoming known (Steimle, 1985; Steimle and Terranova, 1991). Chemical analyses of sediment cores tend to confirm that sediment chemical concentration around the 12-MDS is related to some types of dumping, and that sediment concentrations decrease appreciably in some areas 20 months after cessation of sewage sludge dumping (Zdanowicz et al., 1993[24]). Distribution of the contaminant-sensitive and insensitive benthic invertebrate species assemblages in the New York Bight (Chang et al., 1992) then imply that material dumped at the 12-MDS reached the bottom in the area where it was dumped, entered the food-web at the lower trophic levels of benthic organisms, and ascended to the higher trophic levels of predatory fish species (Steimle et al.[25,26]). Similar food-web dynamics could have influenced the temporal and spatial differences in abundance of commercially important species measured in the principal area of sludge influence at the 106-MDS.

Conclusions

Effects of sewage sludge dumping even in the deep ocean at the 106-MDS, like sludge dumping at the 12-MDS in the New York Bight and release of sewage from outfalls in southern California outfalls, cannot be excluded as a factor measurably affecting fishery resource abundance and composition. Natural factors may have caused the population fluctuation assessed around the 106-MDS, but there are no adequate data sets for testing the likelihood that such phenomena, and not deepwater dumping, are responsible. Data sets are even more inadequate for measuring interactive effects of dumping, fishing, and natural phenomena, which must certainly occur. On the other hand, there are identifiable mechanisms by which changes in abundance and composition of fishery resources could have come about in relatively few years as a result of sludge dumping after prior years of industrial and chemical waste disposal at this deepwater site. Increased contamination of the food-web and environment could well have directly and indirectly, possibly differentially, affected both behavior and reproduction of ecologically linked species leading to the unfavorable temporal, spatial, and seasonal differences of the fishery resources around the 106-MDS.

Analyses of bottom trawl surveys and fishery landings data in relation to environmental pollution are of increasing importance as efforts are made to measure any impact of toxic contaminants on fishery resource. The influence of natural and man-induced environmental factors, such as waste dumping, on fishery resources can be treated as concomitant variables (Thomas et al., 1976; Butler and Schutzman, 1979; Marking and Kimerle, 1979; Geyer, 1981, a and b; Reed et al., 1985; Miller et al., 1988; Wallace et al., 1988; Stoddard and Walsh, 1988; Connor, 1989; Gift et al., 1989; Stanford and Young, 1988, Furness and Rainbow, 1990; Word et al., 1990; Manning, 1991). Interpretation of fishery assessment data in relation to the sewage sludge dumping at the 106-MDS, as developed in the discussion above, makes clear that future efforts in all these regards would benefit from better and updated baseline data on the fishery resources. If there were food-web dynamics data as well as more behavioral and reproductive data on responses of marine fish to contaminant levels known to occur in the ecosystems of concern, we would then be better able to understand and determine causal factors influencing species abundance and fishery resources in the vicinity of the 106-MDS and adjacent continental shelf waters. Much is still to be learned about both the natural fluctuation of environmental factors that influence species abundance and population dynamics and about anthropogenic effects on the natural environment. A better understanding of natural and man-induced effects on fishery resources is necessary to minimize adverse controllable impacts on fisheries resources and requires further synthesis of environmental and fishery assessments.

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Comparison of age at sexual maturity and other reproductive parameters for two stocks of spotted dolphin, *Stenella attenuata*

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Abstract.—We examined the average age at attainment of sexual maturity (ASM) and several other reproductive parameters for evidence of density compensatory responses in two stocks of the spotted dolphin, *Stenella attenuata*. The northern offshore and southern offshore stocks were compared because both have been exploited by the yellowfin tuna purse-seine fishery, but at different rates. We predicted decreasing trends in the ASM and increasing trends in the pregnancy rate for each stock because both have declined in abundance. A lower ASM and a higher pregnancy rate were predicted for the sample from the northern offshore stock because it has been exploited to a greater extent than the southern offshore stock. No statistically significant trends were found in the ASM, but the increase in the proportion of sexually mature females simultaneously pregnant and lactating for the northern offshore stock and the decrease in the proportion of mature females in the population for the southern offshore stock over time were statistically significant. The mean estimate of ASM was significantly higher for the northern offshore stock, 11.1 years (SE=0.236), than for the southern offshore stock, 9.8 years (SE=0.264). No significant differences between stocks were found in the mean estimates of reproductive parameters. Our analyses of temporal trends in several biological parameters did not provide conclusive evidence for compensatory responses having occurred, and therefore some possible explanations are considered.

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Fishery Bulletin 91:611–618 (1993).

As population abundance declines, compensatory responses, such as increases in survival and pregnancy rates and a decrease in the average age at attainment of sexual maturity (ASM), are thought to occur (Eberhardt, 1977; Eberhardt and Siniff, 1977). Each situation must be carefully evaluated to determine whether compensatory responses will be detected because the magnitude of the parameter response and the range of population densities over which measurements are made are likely to be species specific, environmentally variable, and subject to parameter measurement error (Goodman, 1981; Fowler, 1981, 1988; DeMaster, 1984a; York, 1987). For species that are difficult to observe, unbiased estimates of survival rates are difficult to obtain, precluding their use as potential biological indices (Barlow and Boveng, 1991). Reproductive rates, however, are generally more easily measured and, if their relationship to population density is determined, may provide indices of compensatory responses for populations (Perrin and Donovan, 1984). For instance, Barlow (1985) reported that in the spotted dolphin (*Stenella attenuata*), the fraction of mature females pregnant, and the fraction of mature females simultaneously pregnant and lactating, may correlate well with per capita resource availability because these parameters were found to be relatively free of collection biases. The identification of biological indices (i.e., biological parameters that correlate with population abundance) would provide a useful management tool to distinguish between trends in population abundance resulting from exploitation or changes in environmental conditions (Hanks, 1981; Gerrodette and DeMaster, 1990).

The spotted dolphin is an ideal choice for this analysis because a lengthy time series of biological and population abundance data are available. This species is used as a cue by purse-seine vessel operators to find yellowfin tuna in the eastern tropical Pacific (ETP) (Perrin, 1969). Dolphins are incidentally killed during fishing operations, and the spotted dolphin population has had the largest number of animals killed in almost every year since 1959 (Smith, 1983; DeMaster et al., 1992). Two stocks of spotted dolphin, the northern offshore and southern offshore stocks, are recognized as separate management units in the ETP (Perrin et al., 1985). During the first decade of the purse-seine fishery, the 1960's, the large numbers of dolphins killed caused the abundance of these two stocks to decline. The northern offshore spotted dolphin was estimated to be between 35% and 50% of their pre-exploitation abundance (circa 1959) by 1979 (Smith, 1983), while the southern offshore stock was estimated to be at 92–98% of its

pre-exploitation size (circa 1973) in 1979 (Smith[1]). Since 1976, both stocks of spotted dolphin are believed to have declined significantly in abundance (Anganuzzi et al., 1992).

As potential biological indices, we estimated ASM and associated reproductive parameters for two stocks of the spotted dolphin. Although for long-lived mammals, like the spotted dolphin, population growth rates are relatively insensitive to changes in ASM and reproductive rates (Eberhardt and Siniff, 1977; Fowler, 1981; Reilly and Barlow, 1986), these parameters have been correlated with changes in population abundance for other marine mammal species (Fowler, 1987). For instance, a decrease in ASM was correlated with a decline in population abundance for baleen whale stocks (Lockyer, 1984). Similarly, a decrease in ASM for crabeater seals was correlated with an increase in the per capita availability of food resources resulting from the exploitation and subsequent reduction in size of baleen whale stocks (Bengtson and Laws, 1985). Likewise, ASM for the striped dolphin of the western Pacific declined in response to exploitation and reduced population abundance (Kasuya, 1985). Based on the predictions of population responses to changes in density (i.e., increases in ASM and decreases in pregnancy rates as population abundance increases) presented by Eberhardt (1977) and Eberhardt and Siniff (1977), we predicted a decline in the ASM for both the northern offshore and southern offshore stocks of spotted dolphin after 1974 as well as a lower ASM for the more heavily exploited northern offshore stock. Similarly, a higher fraction of sexually mature females pregnant and those simultaneously pregnant and lactating should be observed for the northern offshore stock.

Methods

Since 1968, when the National Marine Fisheries Service (NMFS) first placed observers aboard U.S. vessels to observe fishery activities, life history data has been collected from all cetacean species incidentally killed in the ETP yellowfin tuna purse-seine fishery. Beginning in 1974, life history data collection procedures were standardized, and the original sampling scheme that selectively collected large, female specimens was replaced by a random sampling scheme that selected the first available dead dolphins brought aboard (Perrin and Oliver, 1982). In 1979, the Inter-American Tropical Tuna Commission (IATTC) joined the NMFS in placing observers aboard U.S. vessels and collecting life history data.

The offshore stocks of spotted dolphin are distinguished geographically as being north or south of 1° south latitude (Perrin et al., 1985). The specimens used in our study were collected from these areas, but the sample of northern offshore stock animals was additionally restricted to those animals collected west of 120° west latitude (Fig. 1). The northern offshore stock was sub-sampled because exploitation has not been spatially uniform. The yellowfin tuna purse-seine fishery began primarily as a coastal fishery in 1959 and gradually expanded farther offshore. The Commission's Yellowfin tuna Regulatory Area (CYRA) was established in 1968 by the IATTC and provides a useful boundary that separates the inshore fishery area from the westernmost region of the ETP yellowfin tuna fishery (Peterson and Bayliff, 1985). The area west of the CYRA was not fished until the late 1960's, and then only for a few months a year. Exploitation in this region has been significantly less than inside the CYRA but much more than in the southern area which has been fished only sporadically since the early 1970's (Punsly, 1983). Although spotted dolphin move along the 10°N latitude as well as from the south to the west (Perrin et al., 1979a; Au and Perryman, 1985; Reilly, 1990), owing to the large size of the area, interchange between areas is probably limited. A standardized data collection scheme was established at about the same time as the fishery expanded into the western and southern regions of the ETP, and therefore we anticipated that these two distinct geographic regions would provide the best opportunity for testing potential biological indices.

Female spotted dolphins with complete life history data (i.e., geographic position, total body length, teeth collected, and both ovaries examined) collected between 1974 and 1988 were selected for this study. Few specimens have been collected from the southern offshore stock, and therefore all available specimens were prepared. Specimens from the northern offshore stock were selected randomly by year and the sample sizes distributed as evenly as possible between years so that annual estimates of ASM could be calculated. A maximum of 50 specimens per year was the target for selecting specimens from the northern offshore stock sample because Hohn (1989) showed that a sample size of 50 accurately estimated ASM and reduced the variance from an estimate based on a sample size of 25. Thin sections of the teeth were prepared and aged by one of us (ACM) with techniques described in Myrick et al. (1983). Prior to estimating age, all specimens were numerically coded in random order to disguise the specimen number so that no inference could be made about the stock or collection year for any speci-

[1]Smith, T. D. (ed.). 1979. Report of the status of porpoise stocks workshop (August 27–31, 1979, La Jolla, California). U.S. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Southwest Fish. Sci. Cent., P.O. Box 271, La Jolla, CA 92038. Admin. Rep. LJ-79-41, 120 p.

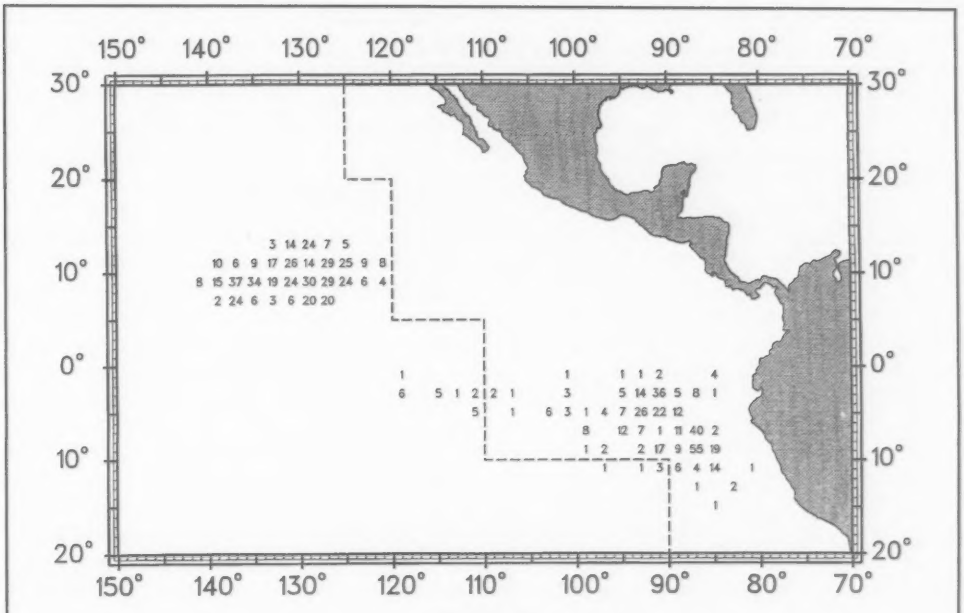

Figure 1

The collection location and sample size of female spotted dolphin specimens selected for this study summarized by 2°-degree square block. The sample of northern offshore spotted dolphins was selected from north of 1° south and west of the Commission's Yellowfin tuna Regulatory Area (the dashed line), and the sample of southern offshore spotted dolphins was selected from south 1° south.

men. The associated life history data were also withheld until each specimen was aged.

ASM and its variance were calculated with the following formula (DeMaster, 1984b; Hohn, 1989).

$$\text{ASM} = x + \sum_{i=x}^{j} p_i$$

$$s^2 = \sum_{i=x}^{j} \{[(p_i * (1-p_i)]/(n_i-1)\},$$

where, x = age of the youngest mature animal,
j = age class of the oldest immature animal,
p_i = proportion immature in age class i,
n_i = sample size for age class i.

Because population abundance estimates are not available for the geographic areas from which our samples were collected, we substituted year as a correlate for evaluating potential biological indices. We tested for temporal trends in ASM with a linear regression to determine whether the slope was statistically significantly different from zero. In addition, data for each stock were pooled for all years, and the null hypothesis of no difference between estimates of ASM for the two stocks was tested with a Student's t-test.

Other reproductive parameters examined include the fraction of the females mature, and the fraction of mature females that were either pregnant or lactating, or simultaneously pregnant and lactating. Trends in these parameters between 1974 and 1988 were tested by using a chi-square test for linear trends (Snedecor and Cochran, 1973), and the mean estimates for each parameter, all years combined, were tested for statistically significant differences with a chi-square test.

Results

Average age at attainment of sexual maturity

ASM was estimated for each year with sufficient data available (Table 1): 1976–1982 and 1984–1988 for the northern offshore stock sample, and 1976, 1979, and 1982 for the southern offshore stock sample. The regression line fit to the data for the northern offshore stock was not statistically significantly different from zero. We did not fit a regression line to the data from the southern offshore stock because only three annual estimates were available. The pooled estimate of ASM for each stock was 11.1 years (SE=0.236; n=520) for

Table 1
Number of samples by year and state of sexual maturity (immature or mature) for the northern offshore and southern offshore stocks of spotted dolphin used to estimate the average age at attainment of sexual maturity.

	Offshore stock			
	Northern		Southern	
Year	Immature	Mature	Immature	Mature
74	0	0	0	0
75	0	0	0	5
76	13	33	41	101
77	16	28	2	6
78	17	27	1	23
79	18	25	16	49
80	15	28	2	9
81	13	24	6	5
82	12	20	42	68
83	9	9	11	11
84	11	27	1	4
85	17	29	0	0
86	18	28	0	0
87	22	26	0	0
88	14	21	0	0
Total	195	325	122	281

the northern offshore spotted dolphin and 9.8 years (SE=0.264; n=403) for the southern offshore spotted dolphin (Fig. 2). This difference in the ASM was statistically significant (t=14.32, df=13, P< 0.001). The range in age of animals attaining sexual maturity was largest for the northern offshore stock. The youngest mature animal was 7 years, and the oldest immature was 16 years. The same range for the southern offshore stock was 6 to 11 years (Fig. 3).

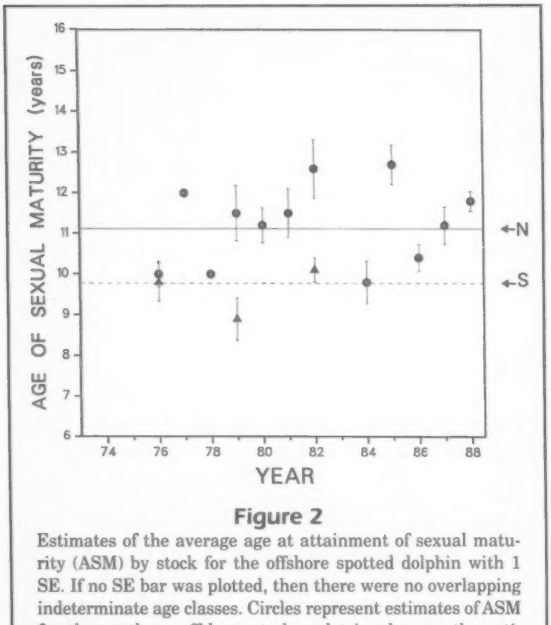

Figure 2
Estimates of the average age at attainment of sexual maturity (ASM) by stock for the offshore spotted dolphin with 1 SE. If no SE bar was plotted, then there were no overlapping indeterminate age classes. Circles represent estimates of ASM for the northern offshore stock and triangles are the estimates for the southern offshore stock. The pooled estimate of ASM for the northern offshore stock (N) is 11.1 years (solid line) and 9.8 years (dashed line) for the southern offshore stock (S).

Reproductive rates

The statistically significant trends in the reproductive parameters examined were an increase in the proportion of mature females simultaneously pregnant and lactating in the northern offshore stock, the predicted compensatory response, and a decrease in the proportion of mature specimens in the sample from the southern offshore stock. When the data for all years were pooled, no statistically significant differences between the mean parameter estimates for the northern offshore and southern offshore stocks were detected (Table 2).

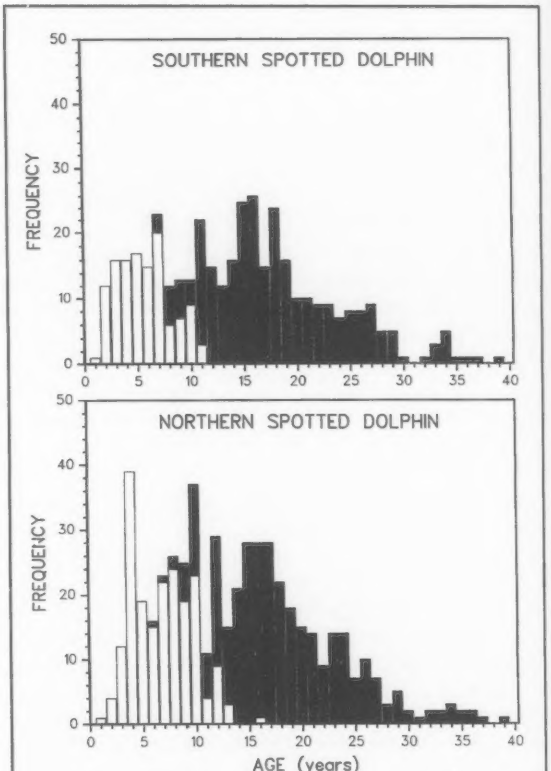

Figure 3
Age-frequency distribution of the northern offshore and southern offshore spotted dolphin specimens aged. The open bars represent the sexually immature females in the sample, and the solid bars represent the sexually mature females in the sample.

Table 2

The mean proportion of females by reproductive condition for the northern offshore and southern offshore stocks of spotted dolphin: the proportion of females in the sample that were sexually mature, and the proportion of sexually mature females that were pregnant or lactating, or simultaneously pregnant and lactating.

Reproductive condition	Stock	
	northern	southern
Mature	0.62	0.69
Pregnant	0.33	0.40
Lactating	0.55	0.47
Pregnant and lactating	0.06	0.06

Discussion

Published evidence of density dependence in dolphin species is rare, and results are inconsistent. For example, in Japan, the striped dolphin, *Stenella coeruleoalba*, is heavily exploited, and the length of the lactation period is reported to have declined (Kasuya and Miyazaki[2]). For exploited spotted dolphins in the western Pacific, a decline in the ASM from 10.1 years in the 1961–63 cohorts to 8.6 years in the 1964–66 cohorts was reported, although the regression coefficient was not statistically significant (Kasuya, 1985). The decline in ASM for female striped dolphin from 9.7 years for the 1956–58 cohorts to 7.4 years in the 1968–70 cohorts was statistically significant (Kasuya, 1985). On the otherhand, for the spinner dolphin (*S. longirostris*) in the ETP, the less exploited southern whitebelly stock has a shorter lactation period than the more heavily exploited eastern spinner dolphin, the opposite of what would be expected for a compensatory response. The annual pregnancy rate, however, is lowest for the southern whitebelly stock and highest for the more heavily exploited eastern stock, as expected for density compensation (Perrin and Henderson, 1984). Barlow (1985) also reported differences in the proportion of mature female spotted dolphins (*S. attenuata*) that were pregnant between the eastern and western Pacific. The lowest proportion of mature females pregnant was observed in the more heavily exploited population, again, contrary to predictions based on the reported exploitation rates and estimates of population abundance for the ETP spotted dolphin. Also, for ETP spotted dolphins, the proportion of lactating mature females significantly increased from 46% in 1973 to 69% in 1981 ($P<0.05$) presumably in response to exploitation; no other statistically significant trends in reproductive parameters were found (Myrick et al., 1986).

Comparisons with other studies are difficult because in most cases the degree of exploitation relative to K has not been quantified, and techniques to estimate age and ASM vary. In our study, statistically significant temporal trends were not detected for ASM but were detected for some of the reproductive parameters. The increase in the proportion of mature females simultaneously pregnant and lactating for the northern offshore stock and the decrease in the proportion of mature females in the population for the southern stock both suggest that the populations are growing. The fact that we did not observe the predicted compensatory responses in ASM may be due to one or more factors, including 1) too few data, 2) parameter estimation or measurement error, 3) a time lag in the response, or 4) biological differences between the stocks. Too few data and environmental periodicity (e.g., El Niño) may increase the variability in annual estimates of parameters, and both factors would reduce the ability to detect differences in potential compensatory responses (Goodman, 1984). The order in which parameters respond to changes in population density is not known, but time lags in responses may be expected owing to the late age at which these animals reach sexually maturity and the multi-year breeding cycle characteristic of the spotted dolphin (Goodman, 1981).

Data for the southern offshore stock of spotted dolphin, in particular, were limited and precluded testing for trends in ASM or the selected reproductive parameters. Although we selected our sample size, a maximum of 50 specimens per year for the northern offshore stock, on the basis of earlier work describing the effect of sample size on the variance for estimates of ASM (Hohn, 1989), our results indicate that larger sample sizes would be required to reduce the variance of the estimates in order to detect small, but biologically significant, changes (e.g., a 1.5-year difference) in ASM. Sample sizes of fewer than 50 per estimate of ASM resulted in too few specimens in the indeterminate age classes (i.e., those age classes with both sexually immature and mature animals) to estimate ASM accurately.

Another concern when analyzing data collected from a kill is whether the age distribution is representative of the population. All immature age classes appear to be underrepresented in both stocks, especially age classes between 10 and 15 years of the northern offshore stock (Fig. 3). This phenomenon was noted in a previous aging study of ETP spotted dolphin but occurred about five age classes earlier than the one we observed (Barlow and Hohn, 1984). This difference may be a result of different readers estimating age,

[2]Kasuya, T., and N. Miyazaki. 1975. The stock of *Stenella coeruleoalba* off the Pacific Coast of Japan. Pap. ACMRR/MM/SC-25 pres. at FAO Scientific Consultation of Marine Mammals, Bergen, February, 1976, 36 p.

although a Kolmogorov-Smirnov test to compare the age-frequency distributions of sexually immature and sexually mature animals from the two studies concluded that the samples were likely drawn from the same population. The underrepresentation of the immature age classes may be a result of segregation in the population by age and sex, fairly typical in large mammal populations, and may affect the estimation of ASM. We investigated the effect of this underrepresentation by assuming a stable age distribution and by apportioning the animals in the indeterminate age classes by sexual maturity under different assumptions of sexual maturity for animals in those age classes, and then calculated ASM. On the basis of segregation by age and sex observed in other species of large mammals, we predicted that the most reasonable scenario for reconstructing the age distribution is that the "missing" animals would be sexually immature. If this indeed were the case, we have underestimated ASM. Under this and other scenarios we investigated, estimates of ASM were consistently over- or under-estimated for both stocks. Therefore, possible sampling biases were unlikely to be responsible for the observed differences in ASM between the northern offshore and southern offshore stocks of spotted dolphin.

The hypotheses we tested required that several implicit assumptions be made, including: 1) constant data collection biases, 2) minimal interchange of animals between areas, 3) consistent estimation of specimen age, 4) linear compensatory response with change in population abundance, 5) constant carrying capacity (K), and 6) equivalent initial life history parameters for the two stocks. The first three of the assumptions are believed to hold reasonably well. Barlow (1985) tested several of the same life history parameters we examined and found them to be relatively insensitive to a number of potential data collection biases; no major changes in the collection of life history data were made after 1974, thus supporting constant data collection bias as a reasonable assumption. Furthermore, the fishery operates in the same areas from year-to-year at approximately the same time of year. In fact, 60% of our sample from the northern offshore stock was collected between July and September, and a further 38% was collected between April and June; in the south, 57% of the sample was collected between January and March, and 27% was collected between October and December. This pattern of sample collection was consistent from year-to-year, and therefore would not affect the pooled or annual estimates of ASM or reproductive parameters. Geographic variability has been noted in earlier studies of spotted dolphin life history data (Hohn and Hammond, 1985; Barlow, 1985); thus selecting specimens from discrete areas as we did reduces the potential for geographic variability to obscure population compensatory responses. Similarly, estimating the age of all specimens at one time and in the blind ensured that age estimates were made as consistently as possible. Information about the validity of assumptions 4) and 5) is not currently available, but violations of the assumptions may provide explanations for our results. There is evidence for differences in the morphological and life history characteristics between the northern offshore and southern offshore stocks of spotted dolphin (see Perrin et al., 1976, 1979b, 1991[3]; Barlow, 1985; Hohn and Hammond, 1985; Myrick et al., 1986; Bright and Chivers[4]) that are likely correlated with the different oceanographic environments of the areas inhabited by these stocks (Au and Perryman, 1985; Reilly, 1990).

We did not find conclusive evidence for compensatory responses in these stocks of spotted dolphin as only one reproductive parameter for each stock showed a statistically significant trend. However, observed trends in both parameters suggest the populations are below K and declining. Our comparison of ASM's suggest further biological differences between the northern offshore and southern offshore stocks or populations. Currently, the order of compensatory responses and their dynamics are not known. In order to be useful as a biological index, a measure of the status of the population must be known and the dynamics of the parameter over a wide range of population densities quantified (Fowler and Siniff, 1992).

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[3]Perrin, W. F., G. D. Schnell, D. J. Hough, J. W. Gilpatrick, and J. V. Kashiwada. 1991. Re-examination of geographical cranial variation in the pantropical spotted dolphin *(Stenella attenuata)* in the eastern Pacific. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Southwest Fish. Sci. Cent., P.O. Box 271, La Jolla, CA 92038. Admin. Rep. LJ-91-39, 46 p.

[4]Bright, A. M., and S. J. Chivers. 1991. Post-natal growth rates: a comparison of northern and southern stocks of the offshore spotted dolphin. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Southwest Fish. Sci. Cent., P.O. Box 271, La Jolla, CA 92038. Admin. Rep. LJ-91-30, 24 p.

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PCR-RFLP analysis on thirteen western Atlantic snappers (subfamily Lutjaninae): a simple method for species and stock identification

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Abstract.—Restriction endonuclease analysis of mitochondrial cytochrome *b* and 12S ribosomal RNA (rRNA) gene fragments amplified by polymerase chain reaction (PCR) was applied to thirteen western Atlantic snapper species (the genera *Lutjanus*, *Ocyurus* and *Rhomboplites*), in order to assess reliability of this method for genetic species and stock identification studies. Eight species (*L. apodus*, *L. buccanella*, *L. campechanus*, *L. cyanopterus*, *L. griseus*, *L. synagris*, *L vivanus* and *R. aurorubens*) could be identified by haplotype analysis on either single or both fragments, while the others (*L. analis*, *L. jocu*, *L. mahogoni*, *L. purpureus* and *O. chrysurus*) were not separated from one another because of overlapping or identical haplotypes observed between species. High nucleon diversity estimates (h = 40–80%) observed within four species (*L. campechanus*, *L. cyanopterus*, *L. griseus* and *L. jocu*) indicated that sufficient intraspecific polymorphism could be detected by the PCR-[RFLP] restriction fragment length polymorphism analysis making it a useful method for investigating genetic stock structure.

In order to manage fisheries resources, it is essential to know the recruitment mechanisms of a given species. Lutjanidae is one of the largest teleostean families, comprising 4 subfamilies, 17 genera, and 103 species (Allen, 1985). More than a half of the lutjanid species belong to the subfamily Lutjaninae in which 14 species under three genera (*Lutjanus*, *Ocyurus* and *Rhomboplites*) have been described in the western Atlantic Ocean. All are fine food fishes and very important for commercial and recreational fisheries, and considerable concern has been focused on the need to understand recruitment in order to manage these reef species (Huntsman, 1981; Doherty, 1983; Roberts and Polunin, 1991). However, the close morphological similarity of larvae among lutjanid species, especially within the subfamily Lutjaninae, has made specific identification of larvae a difficult task (Richards, 1985; Leis, 1987; Richards and Lindeman, 1987). Allozyme electrophoresis has proven useful for identifying some fish species even at their embryonic or larval stages (Morgan, 1975; Smith and Crossland, 1977; Mork et al., 1983; Graves et al., 1989). Since DNA is virtually the same in any cell type of an individual and can be extracted from ethanol-preserved specimens, restriction fragment analyses of DNA is becoming a preferred method to investigate variation and relationships between fish species (Billington and Hebert, 1991). Distinct restriction fragment patterns between three bass species of the genus *Paralabrax* could be detected in ethanol-preserved individual eggs and larvae *via* Southern blot analysis (Graves et al., 1990). Although conventional mitochondrial DNA (mtDNA) methods are powerful for detecting variation in restriction fragment length, intensive DNA analysis would be difficult especially on very small samples, such as fish embryos or larvae, which offer a very small amount of DNA. To overcome this problem, the use of the polymerase chain reaction (PCR) method, which can amplify DNA sequences more than 10-million fold (Saiki et al., 1988), is neccessary. In this paper, we report amplification of two mitochondrial genes (cytochrome *b* and 12S rRNA) using fresh or frozen samples, ethanol-preserved embryos and larvae, and alcohol-preserved museum samples of thirteen western Atlantic snapper species, and results of restriction fragment length polymorphism (RFLP)

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analysis on these two DNA fragments within and between species.

Materials and methods

Sample collection

The species used and sources of collection are listed in Table 1. All of the fresh or frozen specimens were collected in the Miami and Key West, FL, area during 1990 and 1991. Fertilized eggs and hatched larvae of *Lutjanus synagris* were raised at 28°C in 380-L tanks and fed a combination of cultured rotifers and wild-caught zooplankton. The eggs (4 to 6 hours after insemination) and larvae (3 and 9 days old) were fixed with 95% ethanol and kept for two months at room temperature. Two eggs and two larvae were randomly chosen. All of the museum specimens were obtained from the Ichthyology Museum at the University of Miami. The time since preservation ranged from 5 to 42 years and it is likely that most were fixed with formaldehyde first and then transferred to ethanol or isopropyl alcohol (C. R. Robins, Professor, Rosenstiel School of Marine and Atmospheric Science, Division of Marine Biology and Fisheries, Univ. Miami, 4600 Rickenbacker Causeway, Miami, FL 33149-1098, pers. commun.). However, exact records on preservation methods were not available. Samples are labelled as to source, for example, Lcy1 and LcyM1 designate *Lutjanus cyanopterus* fresh or frozen specimen No. 1 and *L. cyanopterus* museum specimen No. 1, respectively.

DNA extraction

Fresh or frozen specimens Mitochondria-enriched or total genomic DNA samples were prepared from fresh or frozen specimens by the method of Chapman and Powers (1984) with slight modification.

Museum specimens A piece of white muscle (0.1–0.2 g) was dissected from each individual, shredded and rinsed in TEK buffer (50 mM Tris, 10 mM EDTA,

Table 1
Sample species of snappers (Lutjaniae: *Lutjanus, Ocyurus* and *Rhomboplites*) and source of collection.

Species	Abbreviation	Fresh or frozen samples locality and date	Museum samples label, locality, and date
L. analis	La	Miami, FL, '90,'91 (6)*	
L. apodus	Lap	Miami, FL, '90,'91 (7)	
L. buccanella	Lb	Miami, FL, '91 (4)	
L. campechanus	Lc	NA	UMML 27092, 28°N, 80°W, '66 (M2)**
			UMML 27093, 28°N, 80°W, '66 (M1)
			UMML 27096, 11°N, 60°W, '66 (M3)
			UM/M2422, Gulf of Mexico, '58 (M4-5)
L. cyanopterus	Lcy	Key West, FL, '91 (1)	UMML15923, Virgin Isl., '59 (M1)
			UM/M4868, Bahama, '56 (M2)
L. griseus	Lg	Miami, FL, '90,'91 (9)	
L. jocu	Lj	NA	Cat.No. 1944, Puerto Rico, '57 (M1)
			Cat.No. 620, Cutler, FL, '49 (M2-4)
			UMML34424, Miami, FL, '86 (M6)
			UM/M5660, Miami, FL, '64 (M7)
L. mahogoni	Lm	Key West, FL, '91 (2)	UMML7523, Knight Key, FL, '57 (M1)
			UM/M4865, Miami,FL '50 (M2)
L. purpureus	Lp	NA	UM/M6093, Venezuela, '65 (M1-2)
			St.5708, Venezuela, '65 (M3)
			St.5658, Venezuela, '65 (M4)
			UM/M6086, Venezuela, '65 (M5-6)
L. synagris	Ls	Miami, Key West, FL, '90 (7)	
L. vivanus	Lv	Miami, FL, '90,'91 (5)	STA5739, Panama, '65 (M1)
O. chrysurus	Oc	Miami, Key West, FL, '90 (8)	
R. aurorubens	Ra	Miami, FL, '90 (6)	

NA=not available.
*=number of individuals examined.
**=museum specimens were labelled, for example, as LcM1 designating *Lutjanus campechanus* museum specimen No. 1. All museum samples were obtained from the Ichthyological Museum of the University of Miami. UMML is the catalogue number for each individual; values in parentheses indicate lots in which several individuals were contained.

1.5% KCl, pH 7.5) for 10 minutes at room temperature. The muscle fibrils were finely minced and homogenized in 5–10 mL of ice-cold TEK buffer by means of a motor-driven, glass-teflon homogenizer. Five or more strokes of the homogenizer were neccessary to grind the toughest muscle tissue. The homogenate was transferred to a plastic centrifuge tube (50-mL capacity), and SDS and proteinase K were added as described in Chapman and Powers (1984). The sample was then incubated at 65°C for 2 hours or more and vigorously shaken at intervals. This crude sample was phenol-chloroform extracted and ethanol precipitated as described in Chapman and Powers (1984). The amount of DNA was usually too small to be seen as a pellet, but was rehydrated in 500 µl of TE buffer (1 mM EDTA, 10 mM Tris-HCl, pH 8.0) and transferred to a 1.5 mL microcentrifuge tube. To concentrate this DNA sample, ethanol precipitation was repeated and the pellet was rehydrated in 10 µl of TE buffer.

Embryos and larvae Each specimen was transferred to a 1.5-mL microcentrifuge tube. TEK buffer was added and decanted several times to rinse specimens and to remove ethanol. The specimen was then homogenized in 500 µl of TEK buffer and total DNA was isolated as described above. The pellet was rehydrated in 10 µl of TE buffer.

Amplification of mitochondrial cytochrome *b* and 12S rRNA genes

The two pairs of primers used that targeted cytochrome *b* and 12S rRNA genes were abbreviated forms of those described by Kocher et al. (1989). The nucleotide sequences of each set of primers were as follows: cytochrome *b*, 5'-GCTTCCATCCAACATCTCAGCATGATG-3' and 5'-GCAGCCCCTCAGAATGATATTTGTCCTC-3'; 12S rRNA, 5'-TCAAACTGGGATTAGATACCCCACTAT-3' and 5'-TGACTGCAGAGGGTGACGGGCGGTGTGT-3'. These primers were synthesized by R. K. Werner in the Department of Biochemistry and Molecular Biology at the University of Miami.

Polymerase chain reaction was carried out in a final volume of 50 µl in a reaction mixture described by Kocher et al. (1989). This reaction mixture was preheated at 94°C for 3 minutes followed by 30–35 cycles of amplification (93°C for 1 min, 44°C for 1 min, and 72°C for 1 min). The same cycle was applied to amplify both cytochrome *b* and 12S rRNA genes. After amplification, the reaction mixture was separated from covering oil and transferred to a 1.5-mL microcentrifuge tube. TE buffer (0.5 mL) was added to the reaction mixture, followed by chloroform: isoamylalcohol (24:1) extraction and ethanol precipitation. After centrifugation at 12,000 × g for 10 minutes, the pellet was dried under reduced vacuum, rehydrated with 10 to 50 µl of TE, and stored at 4°C.

Endonuclease digestion for amplified DNA fragments

Nine restriction endonucleases used were *Alu* I, *Cfo* I, *Dde* I, *Hae* III, *Hin* fI, *Mbo* I, *Msp* I, *Rsa* I, and *Taq* I, all recognizing symmetric 4-base pair sequences. One unit of each enzyme was applied to 1 to 2 µl of amplified PCR product in a final reaction volume of 5 µl. The digested samples were electrophoresed through 2% NuSieve (3:1) (FMC BioProducts, Rockland, ME) agarose gels in TBE buffer (90 mM Tris-boric acid, and 2 mM EDTA). DNA bands were visualized and photographed following electrophoresis and staining with ethidium bromide.

Data analysis The size of fragment amplified and restricted was estimated in comparison with a size standard (1 kb ladder, BRL). Nucleotide sequence divegence (p) (Upholt, 1977) was calculated by using the proportion of shared restriction fragments between specimens. Restriction patterns by each endonuclease were designated A, B or C for composite haplotype analysis, and the number of haplotype was used to estimate nucleon diversity (h) (Nei and Tajima, 1981) within species.

Results

Gene amplification

Each pair of primers successfully amplified 355±5 bp and 450±5 bp fragments of the cytochrome *b* and 12S rRNA genes, respectively. There were no apparent differences in the fragment size among species. Both DNA fragments in all of the fresh and frozen specimens and in ethanol-preserved embryos and larvae were well amplified. In contrast, the amount of DNA was usually much less in museum specimens, to the extent that PCR did not always amplify one or both of the gene fragments.

Restriction fragment analysis

Figure 1, A and B, shows representative restriction patterns with diagnostic enzymes for amplified fragments of the cytochrome *b* and 12S rRNA genes. The restricted fragment distributions for nine enzymes of 72 individuals in cytochrome *b* and 59 individuals in 12S rRNA genes are shown in Tables 2 and 3.

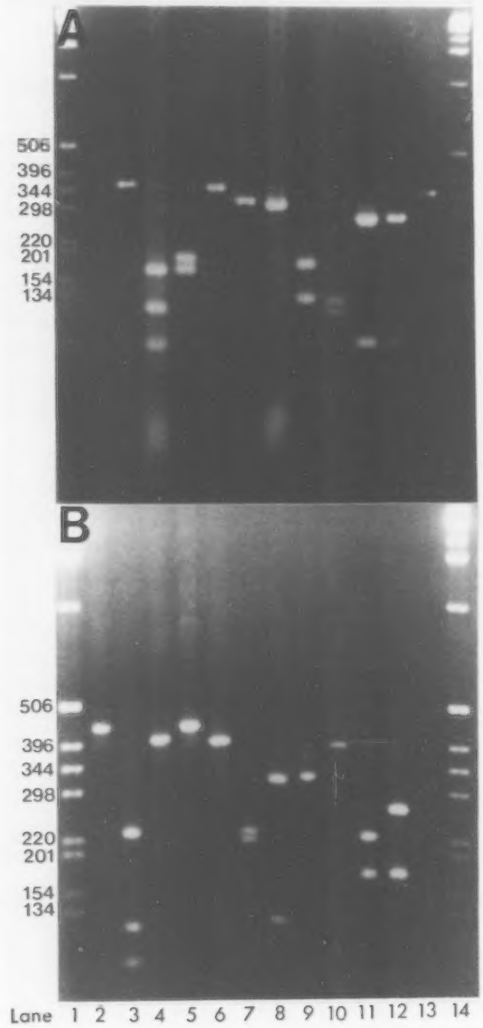

Figure 1

Restriction profiles of cytochrome *b* (**A**) and 12S rRNA (**B**) gene fragments amplified by PCR. (A) lanes 1 and 14 (size standard), 2 (blank), 3 and 4 (La and Ra digested by *Alu* I), 5 and 6 (Lap and Lv digested by *Cfo* I), 7 and 8 (La and Ra digested by *Dde* I), 9 and 10 (Ra and Ls digested by *Hae* III), 11 and 12 (Lap and Ra digested by *Hin* fI), and 13 (La no digestion); (B) lanes 1 and 14 (size standard), 2 (La no digestion), 3 (La digested by *Alu* I), 4 (Lb digested by *Cfo* I), 5 (Ls digested by *Dde* I), 6 (Lg digested by *Hae* III), 7 (Oc digested by *Hin* fI), 8 (Ls digested by *Mbo* I), 9 and 10 (Lg and La2 digested by *Msp* I), 11 and 12 (Lg and La digested by *Taq* I), and 13 (blank).

For the 355 bp cytochrome *b* fragment, four enzymes (*Mbo* I, *Msp* I, *Rsa* I and *Taq* I) had no restriction sites in any of the species. *Dde* I had restriction site(s) in all species examined, without apparent size difference in restricted fragments within and between species. Variation on the restricted fragment length within and between species was observed for four of the nine enzymes examined. *Alu* I appeared to have no restriction sites in this fragment of all *Lutjanus* spp. and *O. chrysurus* (Oc), but two restriction sites producing three fragments (170, 110 and 74 bp) were evident in *R. aurorubens* (Ra). *Cfo* I, *Hae* III and *Hin* fI digestions revealed polymorphism within species; *L. griseus* (Lg) for all three enzymes, *L. analis* (La) for *Cfo* I and *Hin* fI, *L. campechanus* (Lc) for *Hae* III and *Hin* fI, and *O. chrysurus* (Oc) for *Cfo* I only.

All nine enzymes had recognition site(s) in the 450 bp 12S rRNA fragment of at least one species. No length variation of the restricted fragments between species was observed for *Cfo* I and *Hae* III digestions. *Taq* I digestion produced 230 and 170 bp fragments in *L. griseus* (Lg), while 280 and 170 bp fragments were observed in the other species. Intraspecific polymorphism was observed for the other six enzymes; *Alu* I (for Lc, Lcy, Lg, and Lj), *Dde* I (for Lc, Lcy, and Lg), *Hin* fI (for La, Lap, Lcy, and Lj), *Mbo* I (for La, Lc, and Lj), *Msp* I (for La, Lcy, and Lj), and *Rsa* I (for Lc, Lcy, and Lj).

Data of restriction fragment patterns were summarized for haplotype analysis for species discrimination (Table 4). Straightforward diagnostic restriction profiles were observed in the 12S rRNA fragment of *L. griseus* (Lg) digested by *Taq* I and in the cytochrome *b* fragment of *R. aurorubens* (Ra) digested by *Alu* I, without RFLP within species. Haplotype analysis on cytochrome *b* fragment separated *L. cyanopterus* (Lcy) and *L. vivanus* (Lv), and that on 12S rRNA gene fragment separated *L. campechanus* (Lc). *L. apodus* (Lap), *L. buccanella* (Lb) and *L. synagris* (Ls) were descriminated by haplotype analysis on both fragments. In contrast, identical haplotypes were found in five individuals of *L. analis* (La1, 5, 52–54), all of *L. mahogony* (Lm1, 2, M2) and in seven of *O. chrysurus* (Oc1–7), and in two individuals of *L. jocu* (LjM2, M3) and in one of *L. purpureus* (LpM2). One individual of *L. analis* (La2), two of *L. jocu* (LjM1, M7), and one of *O. chrysurus* (Oc50) had their own distinct haplotype.

Mean percent nucleotide sequence divergence ($\bar{p}$) within species ranged from 0 to 2.43 with an average of 0.65. The nucleotide sequence divergence for those between species within the genus *Lutjanus* ranged from 0.11 to 3.84 with an average of 1.69, for those between

Table 2

Restriction profiles of 355 bp cytochrome *b* gene fragment of 13 snapper (Lutjanidae) species. 1=present; 0=absent.

Enzymes and fragments	Species and sample number																		
	La		Lap	Lb	Lc			Lcy	Lg		Lj	Lm	Lp	Ls	Lv	Oc		Ra
	1,5, 52–54	2	1–7	1–4	M1, M2,M3	M4	1, M1,M2	2–5, 8–10,13	6	M1–M3	1,2,M2	M1–M6	1–6, 9	1–5, M1	1–7	50	1–6
Alu I	355	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
	170	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	110	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	74	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Cfo I	355	1	0	1	1	1	0	0	1	1	1	1	0	1	1	0	1
	185	0	1	0	0	0	1	1	0	0	0	0	1	0	0	1	0
	170	0	1	0	0	0	1	1	0	0	0	0	1	0	0	1	0
Dde I	330	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Hae III	190	0	0	1	1	0	1	0	1	1	0	1	0	0	0	0	1
	120	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	100	1	1	0	0	1	0	1	0	0	1	0	1	1	1	1	0
Hin fI	355	1	0	0	0	1	0	1	0	1	1	1	1	0	1	1	0
	275	0	1	1	1	0	1	0	1	0	0	0	0	1	0	0	1
	80	0	1	1	1	0	1	0	1	0	0	0	0	1	0	0	1
Mbo I	355	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Msp I	355	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Rsa I	355	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Taq I	355	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table 3

Restriction profiles of 450 bp 12S rRNA gene fragment of 13 snapper (Lutjanidae) species. 1=present; 0=absent.

		Species and sample number																				
		La		Lap		Lb	Lc		Lcy		Lg		Lj			Lm	Lp	Ls	Lv	Oc	Ra
Enzymes and fragments		1,5, 52–54	2	1–3	7	1–4	M1, M4	M5	1, M1	M2	2,3,5 6,8,9	4	M1	M2, M3	M7	1,2,M2	M2	1–6,9	1–5,M1	1–7,50	1–3
Alu I	450	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0
	230	1	1	1	1	1	1	0	1	0	1	0	0	1	1	1	1	1	1	1	1
	120	1	1	1	1	1	1	0	1	0	1	0	0	1	1	1	1	1	1	1	1
	100	1	1	1	1	1	1	0	1	0	1	0	0	1	1	1	1	1	1	1	1
Cfo I	420	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Dde I	450	1	1	1	1	1	1	0	1	0	1	1	0	1	1	1	1	1	1	1	1
	420	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0
Hae III	410	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Hin fI	450	0	1	1	0	0	1	1	0	1	0	0	1	0	1	0	0	1	0	0	1
	230	1	0	0	1	1	0	0	1	0	1	1	0	1	0	1	1	0	1	1	0
	220	1	0	0	1	1	0	0	1	0	1	1	0	1	0	1	1	0	1	1	0
Mbo I	450	0	1	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0
	320	1	0	1	1	1	1	0	1	0	1	1	0	1	1	1	1	1	1	1	1
	130	1	0	1	1	1	1	0	1	0	1	1	0	1	1	1	1	1	1	1	1
Msp I	450	0	0	0	0	0	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0
	400	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	320	1	0	1	1	1	0	0	1	0	1	1	0	1	1	1	1	1	1	1	1
Rsa I	450	1	1	1	1	1	1	0	1	0	1	1	1	1	0	1	1	1	1	1	1
	250	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0
	120	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0
Taq I	280	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1
	230	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
	170	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table 4

Haplotypes of 74 individuals of thirteen snapper (Lutjanidae) species, and percent sequence divergence (p) and nucleon divergence (h). Eleven columns represent 9 endonucleases: *Alu* I (1 and 5); *Cfo* I (2); *Dde*I (6); *Hae*III (3); *Hin*fI (4 and 7); *Mbo*I (8); *Msp*I (9); *Rsa*I (10); *Taq*I (11).

Gene	cytochrome *b*				12S rRNA								
specimen	1	2	3	4	5	6	7	8	9	10	11	p*	h**
La1, 5, 52–54	A	A	B	A	B	A	B	B	C	A	B		
La2	A	B	B	B	B	A	A	A	B	A	B	1.2	33.3
Lap1–3	A	B	B	B	B	A	A	B	C	A	B		
Lap4–6	A	B	B	B	—	—	—	—	—	—	—		
Lap7	A	B	B	B	B	A	B	B	C	A	B	0.25	28.6
Lb1–4	A	A	A	B	B	A	B	B	C	A	B	0	0
LcM1	A	A	A	B	B	A	A	B	A	A	B		
LcM2,3	A	A	A	B	not amplified								
LcM4	A	A	B	A	B	A	A	B	A	A	B		
LcM5	not amplified				A	B	A	A	A	B	B	1.54	70.0
Lcy1,M1	A	B	A	B	B	A	B	B	C	A	B		
LcyM2	A	B	A	B	A	B	A	A	A	B	B	2.43	66.7
Lg2,3,5,8,9	A	B	B	A	B	A	A	B	C	A	C		
Lg4	A	B	B	A	A	A	A	B	C	A	C		
Lg6	A	A	A	B	B	A	A	B	C	A	C		
Lg10,13	A	B	B	A	—	—	—	—	—	—	—	0.7	41.7
LjM1	A	A	A	A	A	B	A	A	A	A	B		
LjM2,M3	A	A	A	A	B	A	B	B	C	A	B		
LjM7	not amplified				B	A	A	B	C	B	B	2.18	83.3
Lm1,2,M2	A	A	B	A	B	A	B	B	C	A	B	0	0
LpM1,3–6	A	A	A	A	not amplified								
LpM2	A	A	A	A	B	A	B	B	C	A	B	0	0
Ls1–6,9	A	B	B	A	B	A	A	B	C	A	B	0	0
Lv1–5,M1	A	B	B	B	B	A	B	B	C	A	B	0	0
Oc1–7	A	A	B	A	B	A	B	B	C	A	B		
Oc50	A	B	B	A	B	A	B	B	C	A	B	0.13	25.0
Ra1–3	B	A	A	B	B	A	A	B	C	A	B		
Ra4–6	B	A	A	B	—	—	—	—	—	—	—	0	0

— = PCR was not attempted.

*Mean percent sequence divergence (Upholt, 1977): individuals possessing incomplete haplotype were excluded.

**Percent nucleon diversity (Nei and Tajima, 1981): individuals possessing an incomplete haplotype were included in the most common haplotype.

Lutjanus and *Ocyurus* ranged from 0.7 to 3.07 with an average of 1.21, for those between *Lutjanus* and *Rhomboplites* it ranged from 1.24 to 2.81 with an average of 2.16, and for that between *Ocyurus* and *Rhomboplites* it was 2.41. Nucleon diversity estimates (h) within species ranged from 0 to 83.3%.

Discussion

Eight out of thirteen western Atlantic snapper species examined may be identified by PCR-RFLP analysis of the two mitochondrial genes, although further analysis for the intraspecific polymorphism is neccessary for

complete discrimination. Snappers are an important component of both the recreational and commercial fisheries in the Southeastern United States, Gulf of Mexico, and Caribbean. Analysis of the distribution of eggs and larvae for life history and recruitment studies of the fishery has been hampered by the inability to identify eggs and larvae of most snapper species. Our results significantly increase the number of eggs and larvae that can be unambiguously identified. Incomplete separation by our methods for all thirteen species is due to sharing of identical haplotypes between some species, which could be caused by possible occurrence of natural hybrids between snapper species (Domeier and Clarke, 1992). The lack of between species resolution may also be attributed to the fact that the amplified fragments are too short for RFLP analysis, since the number of restriction sites is subjected to the fragment length.

Average sequence divergence observed between the snapper species is also low compared with those between congeneric species of other fish taxa where the entire mtDNA molecule was analyzed (see Billington and Hebert, 1991). Bartlett and Davidson (1991) using direct nucleotide sequence of 307 bp cytochrome *b* gene fragment found several distinct nucleotide substitutions between four tuna species of the genus *Thunnus*. Since many of the nucleotide substitutions observed between these tuna species are found to create or destroy palindromic sequences, PCR-RFLP analysis on the 355 bp cytochrome *b* gene fragment alone could identify these tuna species using only a few endonucleases (Chow and Inoue, 1993). They also found that RFLP analysis of cytochrome *b* and 12S rRNA gene fragments provided insufficient variation to separate all eight species of the genus *Thunnus*, but all tuna species could be separated using a 940 bp fragment of flanking region between ATPase and cytochrome oxidase subunit III genes. Similar analysis on the cytochrome *b* gene fragment could separate six billfish Pacific species and five Atlantic species, but separation of all species was incomplete (Chow[1]).

Although it appears that RFLP analysis may fail to pick up distinct nucleotide substitutions between species, analysis on longer fragments may overcome this disadvantage. Thus, differentiation between closely related species appears to vary among regions within the mtDNA and in a given gene may vary among taxa. Recently, Silberman and Walsh (1992) using PCR-RFLP analysis on the 28S ribosomal RNA gene simply and unambiguously distinguished three spiny lobster species of the genus *Panulirus*. Nuclear rDNA may be better suited for species descrimination, where little intraspecific polymorphism is expected (Dover, 1982).

We have observed relatively high intraspecific polymorphism in some snapper species, in which the nucleon diversity (h) of *L. campechanus, L. cyanopterus, L. griseus,* and *L. jocu* are 70, 67, 42, and 83%, respectively. Gyllensten and Wilson (1987), using restriction analysis on the entire mitochondrial DNA molecule, reported nucleon diversity of brown trout (*Salmo trutta*) to be 72%. Carr and Marshall (1991), using direct nucleotide sequence of a 298 bp cytochrome *b* gene, reported that of the western Atlantic and Norwegian populations of Atlantic cod (*Gadus morhua*) to be 36 and 88%, respectively, comparable with those of some snapper species. It is highly probable that some snapper species contain a large number of maternal lineages within the population, which can be detected by PCR-RFLP analyses as demonstrated in this study.

The present study indicates that PCR-RFLP analysis is much simpler and less expensive than conventional mtDNA and nucleotide sequence analyses and better suited for large-scale genetic surveys requiring large numbers of specimens. With this method, not only are quantitative analyses of species composition at early life history stages possible, but also intensive genetic analysis. Since relatively little is known about the spawning habits, dispersal, and migration of these snapper species, it would be very interesting to compare, for example, genetic structures between adults and their early life stages. Such genetic analyses would aid in evaluating the utility of management schemes, such as reserves and protected areas.

[1]Chow, S. 1992. Identification of billfish species using mitochondrial cytochrome *b* gene fragment amplified by polymerase chain reaction. ICCAT Working Doc. SCRS/92/66.

Acknowledgments

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Effects of dolphin group type, percent coverage, and fleet size on estimates of annual dolphin mortality derived from 1987 U.S. tuna-vessel observer data

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Abstract.—Amendments enacted in 1988 to the U.S. Marine Mammal Protection Act of 1972, coupled with changes during the 1980's in the composition of the international purse-seine fleet fishing "on dolphin" for yellowfin tuna in the eastern tropical Pacific (ETP), motivated a simulation experiment to assess the effects of fleet size and observer coverage level on estimates of cumulative annual mortality for dolphin stocks, where data quality differed greatly between stocks. Results from this simulation study provided a basis for subsequent U.S. legislation regarding criteria for comparing dolphin mortality rates of U.S. vs. non-U.S. fleets fishing in the ETP, pursuant to regulations governing import of tuna from this area.

Accuracy and precision of mortality estimates were influenced very strongly by data quality (dolphin group type) and moderately strongly by fleet size and level of observer coverage. Because neither fleet size nor dolphin group type can be controlled by a sampling program, desired levels of accuracy and precision in estimates of dolphin mortality can be achieved only by manipulating the level of observer coverage. The unexpectedly strong effect of dolphin group type implies that to protect all groups equally, observer levels should be chosen to accommodate the dolphin group type exhibiting the poorest-quality data.

U.S.-registered tuna purse-seiners in the eastern tropical Pacific Ocean (ETP) inadvertently kill dolphins during fishing operations (DeMaster et al., 1992). Since 1972 this kill has been monitored by scientific observers accompanying seiners on routine fishing trips. The kill data are monitored to determine whether annual quotas have been reached for dolphin mortality overall, and for several specific stocks of dolphins. If any quota is reached, fishing must cease on the affected stock for the remainder of the calendar year. Since 1989, the data have also been used to determine annual dolphin mortality rates for comparison with non-U.S. fisheries[1].

Prior to 1987, this kill monitoring was accomplished by observers on 30–50% of the trips made by U.S. purse-seiners during a given year. Thus, mortality was estimated rather than measured directly. In the absence of any alternative (i.e., higher rates of observer coverage), the accuracy and precision of these estimates were assumed to be adequate. This assumption was acceptable at the time because the U.S. fleet was large and generated many data. About 100 vessels fished the eastern tropical Pacific Ocean (ETP) each year, each vessel making two to five trips of about three-months duration each.

The situation changed dramatically in the 1980's when, for a variety of reasons (Sakagawa, 1991), the U.S. fleet began to decrease 20–30% per year, decreasing from over 90 vessels in 1981 to about 10 vessels in 1992. This decrease in size of the U.S. fleet was offset by a reciprocal increase in number of non-U.S. vessels fishing in the ETP. This increase has been dominated by Mexican vessels (fleet size exceeding 50 vessels by 1990) but also includes smaller fleets from several other nations. These smaller fleets range in size from 1 to about 20 vessels (IATTC Annual Reports, 1980–1991). In addition to these changes in composition of the international fleet, amendments passed in 1988 to the U.S. Marine Mammal Protection Act stipulated that yellowfin tuna caught in the ETP could be imported from non-U.S. countries only if mortality rates for those countries were comparable to U.S. kill rates.

These events created a strong incentive to evaluate the effects on mortality estimates of varying observer coverage levels and fleet sizes. Because the new regulations pertained to some individual stocks in addition to dolphin mortality overall, the previously uninvestigated effect of dolphin group type was also of interest.

[1] The mortality estimator used in these simulations (kill/day) is not the same estimator used currently (1993) to determine mortality comparability (kill/set). The reasons for this appear in the methods section.

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The U.S. data set for 1987 provided a unique opportunity to investigate these effects. That year, for the first time, observers accompanied nearly 100% of trips by U.S.-registered purse-seiners to the ETP. By subsampling the 1987 data set, various observer levels and fleet sizes could be simulated and their effects on dolphin mortality estimates studied. In the absence of any other available data, these data were taken by NMFS as roughly representative of the international fleet. The simulation results guided subsequent legislation regarding mortality comparability criteria for non-U.S. fleets in the ETP.

Total annual mortality estimates are presented here for three dolphin groups (all stocks combined): spotted dolphin (*Stenella attenuata*); whitebelly spinner dolphin (*Stenella longirostris longirostris*); and common dolphin (*Delphinus delphis*). These three groups represent the range of data types (good, intermediate, and poor in quality) in the 1987 data set, and are generally representative of data types collected every year that may be affected by variable fleet sizes and levels of observer coverage in future fishing years.

Accuracy and precision of the mortality estimates are presented in terms of relative bias and coefficient of variation. Frequency distributions and analytic confidence intervals are examined for underlying patterns in mortality data responsible for generating observed patterns in accuracy and precision.

Methods

Data

During 1987, 34 U.S.-registered purse-seine vessels carrying certificates that permitted fishing "on dolphins" made a total of 134 fishing trips to the eastern tropical Pacific Ocean (ETP). Of these vessels, 33 made 124 trips that were observed by either a National Marine Fisheries (NMFS) or an Inter-American Tropical Tuna Commission (IATTC) technician. These 124 observed trips comprised the entire population of trips for this study. They accounted for 93% of all trips and 96% of all days spent at sea in the ETP by U.S.-registered tuna purse-seiners during 1987. Trips made by individual vessels within fleets were considered replicates and observed trips were assumed to represent unobserved trips accurately. Data consisted of records of date, time, trip number, set number, and kill per set.

Between 4 and 20 vessels carrying observers departed each month during 1987 (Fig. 1). Approximately the same number (although not necessarily the same vessels) of observed vessels (about 30) fished in the ETP during any given month (Fig. 2). Just over half the trips (65/120) lasted about three months. Trip duration ranged from one to six months (Fig. 2). Number of trips incurring mortality of each dolphin group type ranged from 93 (offshore spotted dolphins; Fig. 3) to 12 (common dolphins).

Dolphin mortality data were not stratified geographically, despite the demonstrated efficiency of this stratification scheme (e.g., Hall and Boyer, 1986) because it can be applied only when data sets provide reasonable numbers of reasonably homogeneous data per stratum. Data tend to be so variable for the less frequently killed groups, such as common dolphins, that mortality estimates tend to be very imprecise (and infrequent) even without stratifying data. This is likely to be a problem for all dolphin types for small fleets in every year[2].

Data quality differed between group types in both frequency and extent of kill. Data for offshore spotted dolphins were the "best" in terms of being most plentiful and most evenly distributed between trips collecting data. The frequency distribution of kill per day per trip (i.e., number of dolphins killed per number of days at sea, for a given trip) is relatively smooth (Fig. 3). Most trips killed about 0.5 dolphins per day, a few

[2]At the time this study was conducted (1988–1989), there were no legal requirements for data stratification. Subsequent to this study, the U.S. Congress enacted legislation (Federal Register Notice Vol. 54, #43, Tuesday March 7, 1989; 54 FR9438-9451) requiring stratification of data for annual mortality estimates into three geographic strata and two dolphin groupings (common dolphins, and all other dolphins).

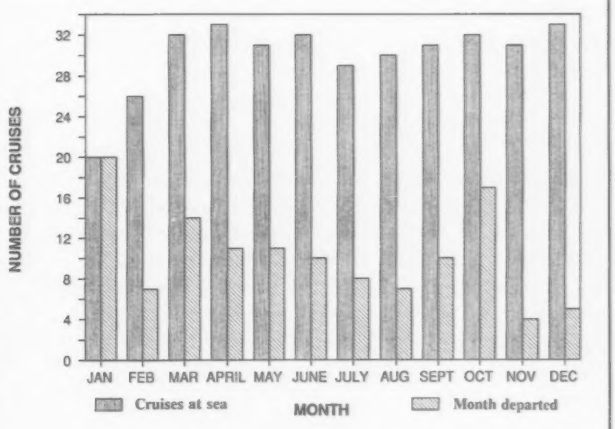

Figure 1

Number of observed U.S.-registered tuna purse-seine vessels at sea, and number of observed vessels leaving port each month during 1987.

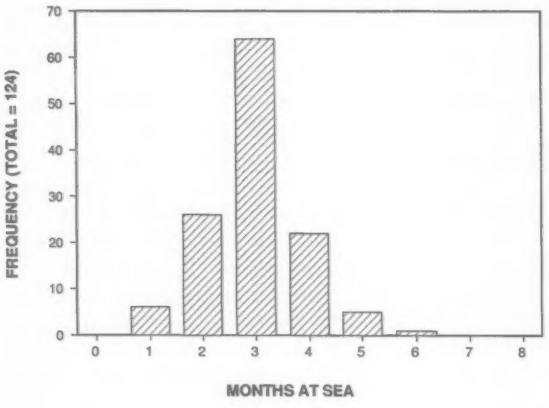

Figure 2
Months spent at sea per trip by U.S.-registered tuna purse-seine vessels carrying observers during 1987.

trips killed no spotted dolphins, and trips with higher kills occurred with decreasing frequency. Data for whitebelly spinner dolphins were intermediate in quality. They were fewer but still relatively smoothly distributed. About half the trips killed no whitebelly spinner dolphins, but of those incurring mortality, most trips were responsible for about 0.1 dolphins per day. Higher kills again occurred with decreasing frequency, with little evidence of extreme outliers. Data for common dolphins exhibited the worst quality, being both sparse and very unevenly distributed. Of the 12 trips incurring mortality of common dolphins, 9 killed about 0.5 dolphins per day, 2 trips killed 1–1.5 dolphins per day, while the remaining trip was responsible for about 9 deaths per day. That one trip was responsible for 67% (594/882) of U.S.-caused common dolphin deaths during 1987.

Simulations

We first selected randomly and without replacement 5, 10, or 20 vessels from the total of 33 observed vessels (Fig. 4). We then sampled randomly and without replacement, 25%, 50%, or 75% of the trips made by each of these selected vessels, without regard to actual timing of those trips throughout the year. We did not force temporal stratification on these small fleet simulations because fleet sizes of 5 or 10 vessels generated so few data.

Vessel selections were replicated 10 times at each combination of fleet size and percent coverage. Trip selections were replicated 50 times from each of the 10 sets of selected vessels. Thus, selected vessels were the same within each set of 50 replicate samples of trips but differed between sets of 50 replicates. Trips conducted by selected vessels in each sample fleet represent the "population" of trips for that fleet replicate.

Each set of 50 replicate selections of trips from a given set of selected vessels generated 50 estimates of cumulative annual mortality (cumulative mortality through December 31). For each set of 50 estimates, we calculated the mean estimate, the coefficient of variation for the mean estimate, and the relative bias of the mean estimate. Thus for each combination of fleet size and percent coverage we generated 10 averaged estimates of annual mortality, 10 coefficients of variation, and 10 estimates of relative bias.

Because the "true" kill varied between fleet replicates depending on the particular vessels and trips selected, the average of these 10 averaged estimates (from the 50 replicates) of annual mortality is not a particularly useful measure in this study. This average represents only the average of the 10 sets of vessels that happened to be chosen for the 10 replicates of fleet size. However, the coefficients of variation and estimates of relative bias for each of the 10 averaged estimates *are* relevant indicators of the ability of the estimation process to precisely or accurately, or both, reflect the true kill, whatever it happens to be for a particular sample of vessels. Accordingly, we present results only for the averages of the 10 estimates of CV and RB generated for each combination of percent coverage, fleet size, and dolphin mortality type. We do not discuss directly the individual mortality estimates.

Estimates

The estimates in this simulation study were derived by using the ratio estimator kill per day, rather than the more precise estimator kill per set (Lo et al., 1982; Hall and Boyer, 1986) because at the time this study was designed and executed, kill per day was still the estimator of choice for NMFS[3].

[3]Kill/day was used by NMFS up until 1987 when 100% observer coverage began, because the quota system required that dolphin kills be monitored continuously throughout the year rather than simply summed at the end of the year. Estimates based on kill/day can be made on a reasonably real-time basis because the day that a vessel leaves port is a readily available datum, and observers report kills by radio on a biweekly basis throughout each observed trip. Number of days fished by the entire fleet (observed plus unobserved) can then be determined relatively simply by summing the number of days at sea since leaving port, and total kill estimated as the product of total fleet days times the estimator kill/day. Biweekly estimates based on kill/set are not possible because observers are not permitted to report set data over the radio. Set data do not become available until after vessels have returned to port.

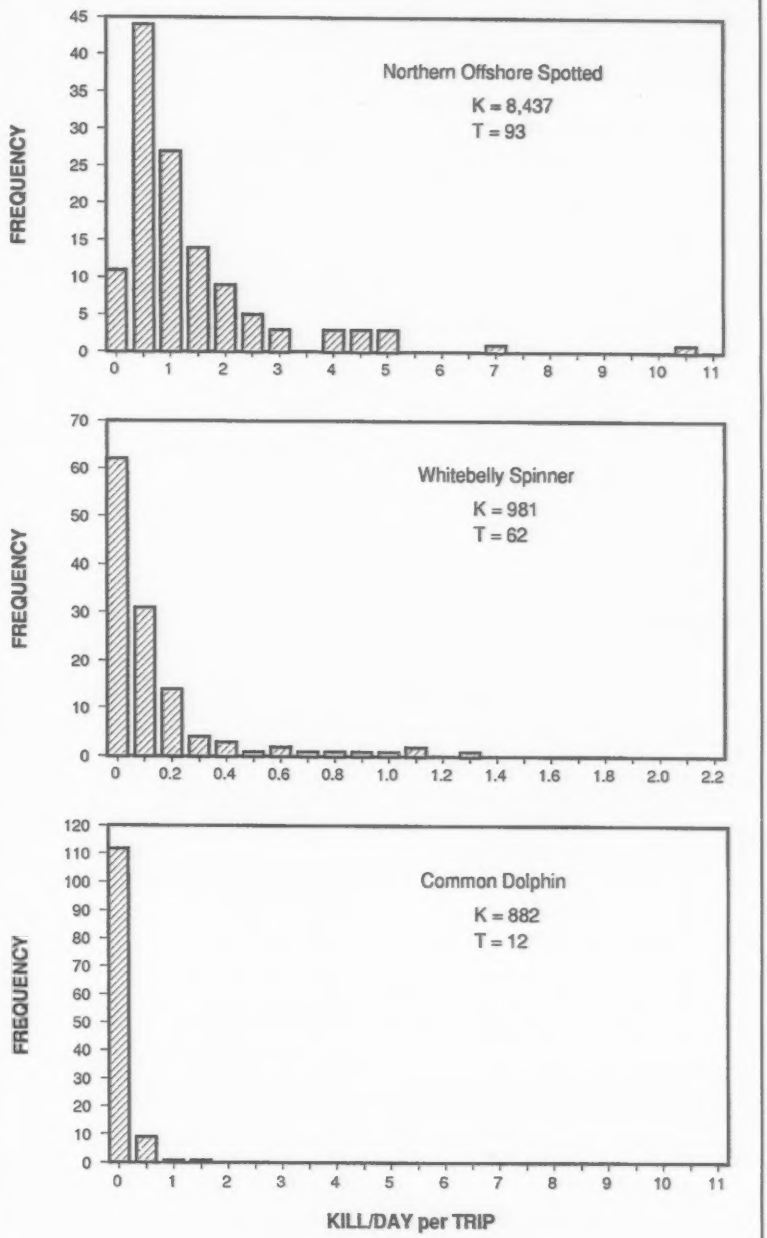

Figure 3

Kill per day during observed trips (124 trips total) by 33 U.S.-registered tuna purse-seiners fishing in the ETP during 1987, for three species of dolphins representing the range of mortality data types. Scales of ordinates and abscissas differ between panels. *K* is total observed kill during 1987; *T* is number of trips incurring kill.

Factors and levels included dolphin group$_i$ (i=1,2,3; offshore spotted, whitebelly spinner, and common dolphin), fleet size$_j$ (j = 1,2,3,4; for 5, 10, 20, or 33 boats), fleet replicate$_k$ (k=1,..,10), percent coverage$_l$ (l=1,2,3; for 25%, 50%, and 75%) and percent coverage replicate$_m$ (m=1,...,50).

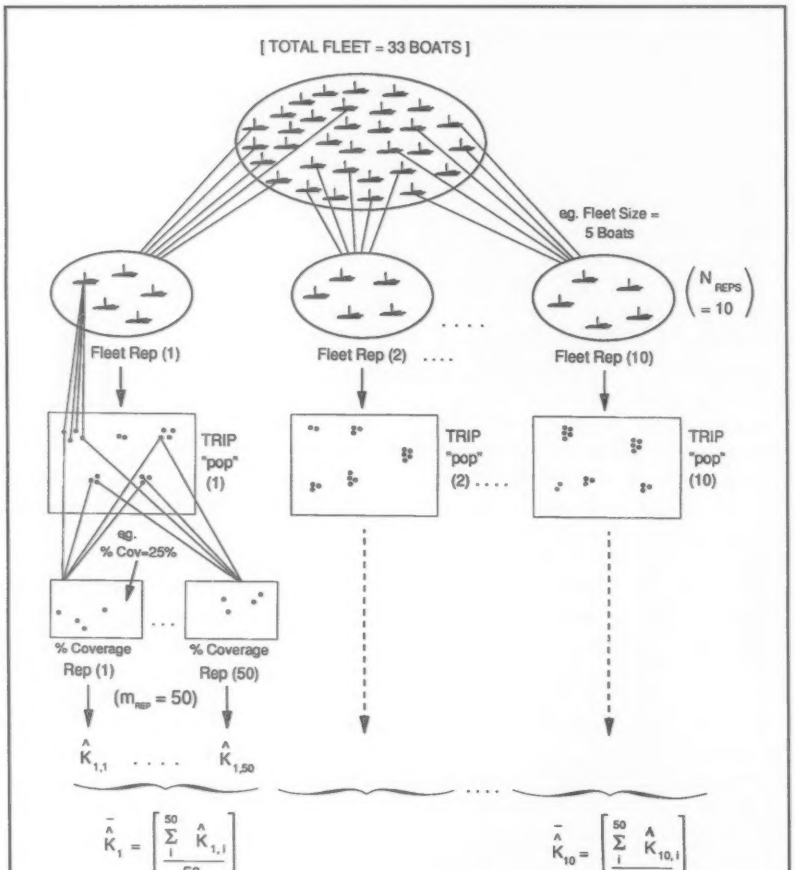

Figure 4
Sampling procedure for simulation study.

Within each replicate, cumulative annual mortality for a given group of dolphins (*YHAT*) is estimated as

$$YHAT_{ijklm} = (K_{ijklm} / DAYS_{jklm}) * TFDAYS_{jk}$$

where K_{ijklm} is the observed cumulative annual kill on all selected trips, $DAYS_{jklm}$ is the observed number of days, and *TFDAYS* is the total number of days spent at sea by all vessels selected for that replicate.

For each set of 50 replicate estimates of annual mortality for a given fleet size, percent coverage, and dolphin group type, we calculated the average estimate ($YAVE_{50}$), the relative bias of that estimate (RBY_{50}), and the coefficient of variation of that estimate (CVY_{50}) as follows

$$YAVE_{50ijkl} = (1/50) * \sum_{m=1}^{50} YHAT_{ijklm}$$

$$RBY_{50ijkl} = 100 * (YAVE_{50ijkl} - known\ kill_{ijk}) / known\ kill_{ijk}$$

and

$$CVY_{50ijkl} = [\sqrt{MSE(YAVE_{50ijkl})}] / known\ kill_{ijk}] * 100$$

where

$$MSE(YAVE_{50ijkl}) = \sum_{m=1}^{50} (YHAT_{ijklm} - known\ kill_{ijk})^2$$

and

$$BYAVE_{50ijkl} = YAVE_{50ijkl} - known\ kill_{ijk},$$

where $BYAVE_{50ijkl}$ is the bias of $YAVE_{50}$, and $V(YAVE_{50ijkl})$ is the variance of $YAVE_{50ijkl}$, calculated as

$$V(YAVE_{50ijkl}) = (1/49) * \sum_{m=1}^{50} (YHAT_{ijklm} - YAVE_{ijkl})^2$$

(Cochran, 1977).

The preceding equations produce 10 $YAVE_{50}$'s, 10 RBY_{50}'s, and 10 CVY_{50}'s for each combination of fleet size, percent coverage, and dolphin group type.

The average of these 10 RBY_{50}'s is

$$RBY_{10ijk} = (1/10) * \sum_{k=1}^{10} (RBY_{50ijkl}),$$

and the average of the 10 CVY_{50}'s is

$$CVY_{10ijk} = (1/10) * \sum_{k=1}^{10} CVY_{50ijkl}.$$

Sampling distributions and confidence intervals

To facilitate interpretation of patterns seen in relative bias and coefficients of variations, we plotted both frequency distributions and analytic 95% confidence intervals for an arbitrarily selected single set of 50 individual replicate estimates of mortality derived under various combinations of conditions. The frequency distributions and confidence intervals illustrate, in particular, variations due to differences between replicates in the fleet selected, in contrast to the relative bias and coefficient of variation, which pertain to sampling properties of the estimator. Only one set of 50 replicates, of the 10 sets generated under each combination of fleet size and coverage level, is illustrated for each combination because the general messages conveyed by the figures were the same for all sets.

Frequency distributions and confidence intervals are plotted only for the cases of 5 and 20 boats at 25% and 75% coverage. Combinations of these values spanned the range of fleet sizes investigated and enabled us to examine whether problems might occur even with coverage as high as 75% when fleet size is as small as 20 (or worse, 5) boats.

The frequency distributions of the 50 estimates illustrate graphically the influence of various combinations of fleet size and observer coverage level on the behavior (dispersion) of the estimator itself (kill/day). The analytic confidence intervals for the 50 estimates illustrate the influence of various combinations of fleet size and observer coverage level, on the estimated precision that may be associated with any individual estimate.

The analytic confidence intervals for individual replicate estimates of mortality were calculated by the International Mathematical and Statistical Library (IMSL) routine SMPRR for ratio estimates (IMSL, 1987). This routine calculates confidence intervals for ratio estimates using the analytic formula for approximate variance of a ratio. The procedure is based on the assumption that a normal approximation to the ratio variance is appropriate (Cochran, 1977; IMSL, 1987). Where data are sparse (fewer than 30 data points in the data set; i.e., in most of the cases in these simulations) this assumption is generally inappropriate (e.g., Cochran, 1977), but for single replicates we had no computationally simple alternative. Bootstrapping confidence intervals for these individual replicates would have eliminated any need for a normal approximation but would have required significantly more computer time to convey essentially the same gross patterns and general message.

Results

Relative bias (RB)

RB was generally small overall, but exceeded the management objective of 5% for common dolphins and whitebelly spinner dolphins when coverage was low (25%; Fig. 5).

Coefficients of variation (CV)

CV decreased with increasing percent coverage and with increasing fleet size in both the "best" data group (offshore spotted dolphin; Fig. 6) and in the "intermediate" data group (whitebelly spinner dolphin). CV decreased with increasing percent coverage but showed no consistent effect of fleet size in the "worst" data group (common dolphin).

Sampling distributions

Frequency distributions were affected somewhat by fleet size (primarily by shifting the central tendency), noticeably by percent coverage (primarily by decreasing the spread of the distribution), and very strongly by dolphin group type (primarily in terms of the number of modes in the distributions; Figs. 7, 8, and 9). Bias and variability increased with small sample sizes and non-smooth data distributions.

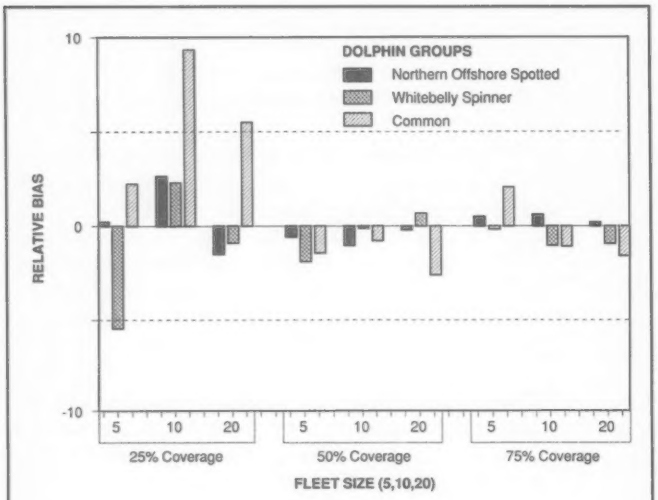

Figure 5

Average relative bias (%) in estimates of annual dolphin mortality as a function of fleet size and percent observer coverage. Dashed line at +5% and –5% indicates management target.

Distributions of mortality estimates for offshore spotted dolphins tended to be relatively narrow and unimodal for fleets of both 5 and 20 vessels, at high coverage (75%; Fig. 7). Distributions remained unimodel but were more dispersed, at low coverage (25%). Distributions for whitebelly spinner dolphins were also unimodal in general but tended to be more dispersed than was the case for offshore spotted dolphins (Fig. 8). Distributions for common dolphins were markedly bimodal and dispersed under all sampling conditions, reflecting the selection (or not) of the one trip with unusually high kill (Fig. 9).

Modal values of mortality estimates for all three dolphin types increased with increasing fleet size (not surprisingly, because more boats generally kill more dolphins) regardless of coverage level. Dispersion also increased with fleet size, more obviously when observer coverage was low than when coverage was high, as more data became available for analysis.

Confidence limits

Confidence limits were affected similarly to frequency distributions. Limits were affected most strongly by dolphin group type, very noticeably by percent coverage, and less strongly but still noticeably by fleet size (Figs. 10, 11, and 12).

Mortality estimates were most stable and confidence intervals dramatically reduced for offshore spotted dolphins at high (75%) observer coverage (Fig. 10). Mortality estimates were more variable and confidence intervals wide and ragged, even with a relatively large fleet (20 boats), for offshore spotted dolphins at low observer coverage (5%). Intervals were most unstable at the combination of lowest observer coverage and smallest fleet size[4]. Confidence interval patterns for whitebelly spinner dolphins were intermediate, being more variable than patterns for northern offshore spotted dophins but being less variable than patterns for common dolphins (Fig. 11). At low coverage and small fleet size, confidence intervals for whitebelly spinner dophins showed the same bimodality characteristic of confidence intervals for common dolphins under all conditions. At high coverage, confidence intervals showed the same relatively stable and

[4]The irregular pattern of the confidence intervals observed for mortality estimates from small fleets (e.g., 5 boats) with low coverage (e.g., 5 boats, 25% coverage) are not surprising because this combination of fleet size and percent coverage means that the mortality estimates are being derived from about 4 observed trips during an entire year (assuming 5 boats make 3 trips/year, 15 trips × 0.25 = 4 trips).

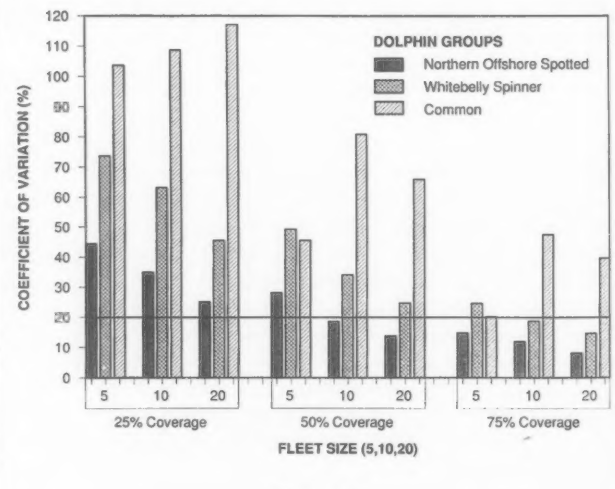

Figure 6

Coefficient of variation (percent) in estimates of annual dolphin mortality as a function of fleet size and percent observer coverage. Dark horizontal bar at 20% indicates management target.

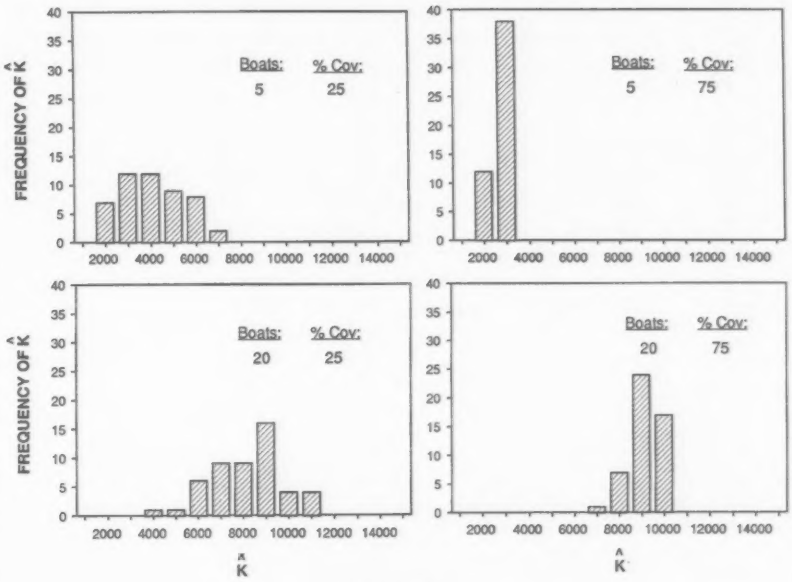

Figure 7

Effect of percent coverage and fleet size on frequency distributions of annual mortality estimates for **offshore spotted dolphins**, from one set of 50 replicates.

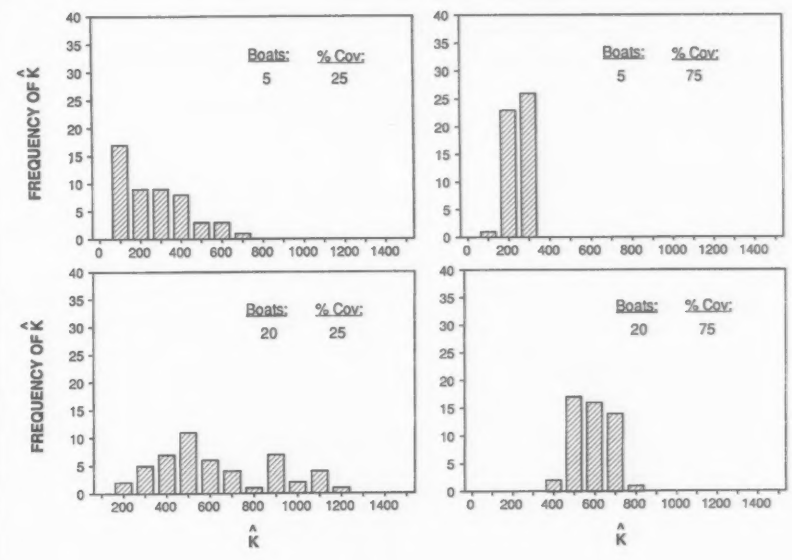

Figure 8

Effect of percent coverage and fleet size on frequency distributions of annual mortality estimates for **whitebelly spinner dolphins**. from one set of 50 replicates.

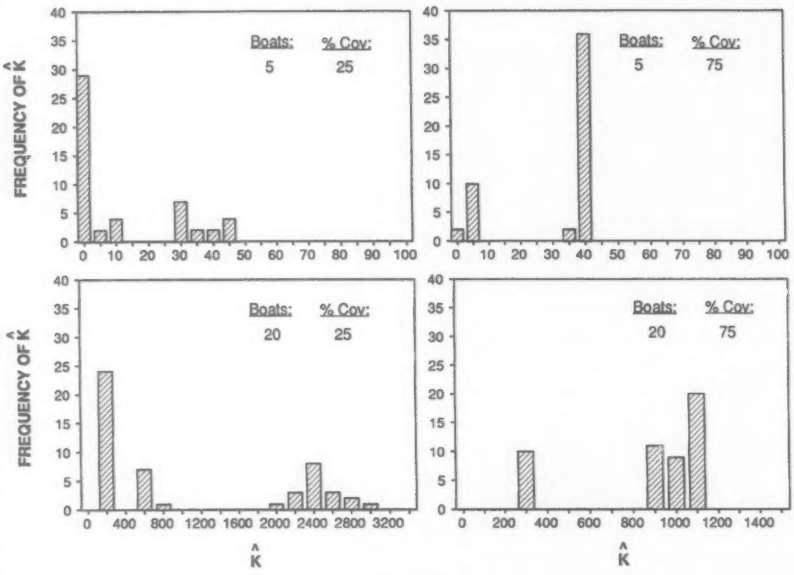

Figure 9

Effect of percent coverage and fleet size on frequency distributions of annual mortality estimates for **common dolphins**, from one set of 50 replicates.

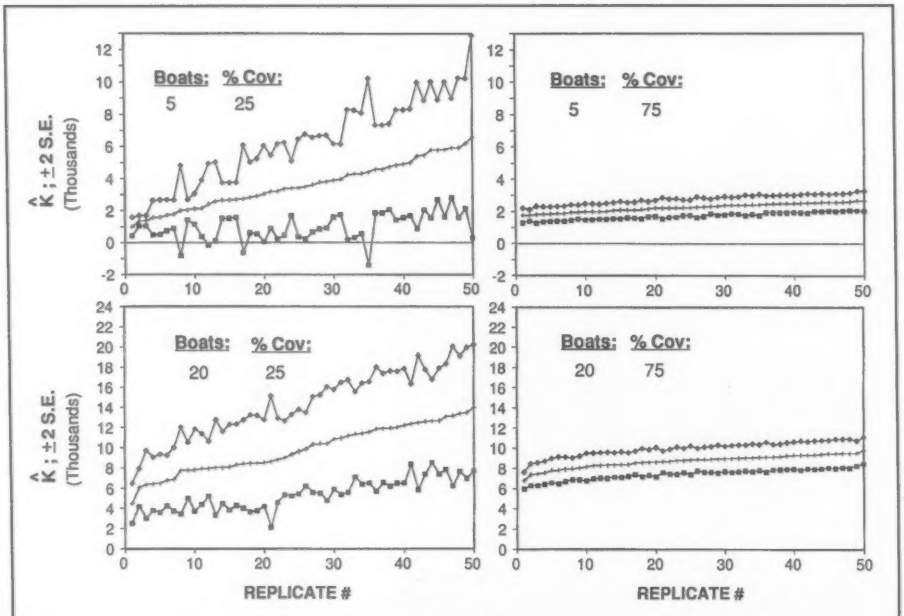

Figure 10

Effect of percent coverage and fleet size on analytic confidence intervals of replicate annual mortality estimates for **offshore spotted dolphins**. Replicate estimates sorted by estimate level, from one set of 50 replicates. Note differences between panels in Y scale.

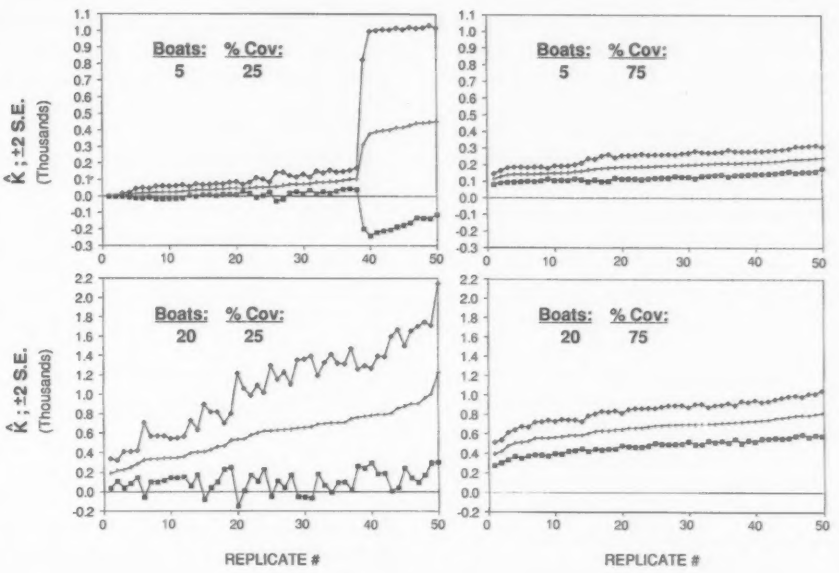

Figure 11

Effect of percent coverage and fleet size on analytic confidence intervals of replicate annual mortality estimates for **whitebelly spinner dolphins**. Replicate estimates sorted by estimate level, from one set of 50 replicates. Mortality estimates expressed in thousands. Note differences between panels in Y scale.

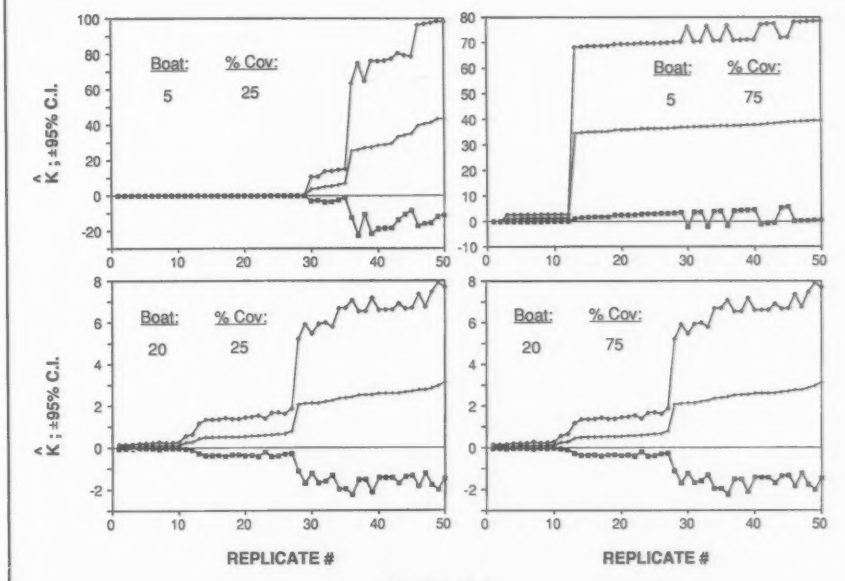

Figure 12

Effect of percent coverage and fleet size on analytic confidence intervals of replicate annual mortality estimates for **common dolphins**. Replicate estimates sorted by estimate level, from one set of 50 replicates. Mortality estimates expressed in thousands. Note differences between panels in Y scale.

narrow limits seen for offshore northern spotted dolphins at high coverage. Confidence intervals for common dolphins illustrate the problems inherent in relatively heterogeneous data, where mortality is sometimes quite high, usually relatively low, and data overall are relatively few. For this species, confidence intervals were extremely variable even with relatively high coverage (Fig. 12) and were very narrow (or nonexistent) for replicates which fortuitously included only low-kill data, but very wide for replicates including a few very high kills.

Although these analytic confidence intervals are clearly inappropriate measures for precise estimation of variance characteristics of the estimates (note negative intervals in some cases; Figs. 10, 11, and 12), they are presented here because they provide an effective illustration of the effects of varying conditions on the variability of the mortality estimates. Despite the inter-group differences, the general response to increasing coverage and increasing fleet size is similar in all three dolphin group types. Confidence intervals become narrower and more stable as more data become available.

Discussion

Dolphin group type

Of the three factors investigated here (dolphin group type, observer coverage level, and fleet size), dolphin group type had the greatest effect on dolphin mortality estimates, followed by percent coverage and fleet size. This hierarchy of effects is controlled by two characteristics of the kill data for each dolphin group type—*frequency* (the number of times that mortality occurs) and *variability* (differences between times in the number of dolphins killed). The *total* number killed can have relatively little influence on the quality of the estimate. This is illustrated by comparing results for whitebelly spinner dolphin and common dolphin. Although total kill was comparable for both dolphin group types (981 deaths of whitebelly spinner dolphin, 882 deaths of common dolphin; Fig. 3) the data sets differed markedly both in number of trips incurring kill (62 for whitebelly spinner dolphin, 12 for common dolphin) and in the distribution of kill per day among those trips (Fig. 3).

The data set for common dolphins exhibits the worst of both characteristics; frequency of kill was low (few trips killed common dolphins) and variability between trips in kill per day was high. These problems exemplify an unfortunate interaction between data collection problems and the ecology of the dolphins themselves. Mortality of common dolphins due to the U.S. fleet during 1987 was *infrequent* because the geographic range of this species is relatively limited and occurs primarily within Mexico's Exclusive Economic Zone. U.S. vessels rarely fish in this area, therefore common dolphins rarely die in U.S. tuna nets in this area. Mortality was *variable* at least in part because common dolphins have an unfortunate habit (in this context) of forming very large schools, pre-disposing them to the possibility of very large-kill "disaster" sets.

The data set for whitebelly spinner dolphin exhibits a problem with only one of the characteristics; data are relatively infrequent. Unlike the case for common dolphins, kill per day was not extremely variable. This similarity in kill per day generates statistics for whitebelly spinner dolphin that are much less biased and variable than for common dolphin.

Observer coverage level

The effect of observer coverage level is influenced both by fleet size and dolphin group type. To achieve a desired level of precision and accuracy in mortality estimates, observer coverage levels will have to be higher in smaller fleets because the available data will be fewer, and higher in dolphin groups with "messy" data, because observer coverage levels affect the probability of encountering an unusually large kill[5].

Observer coverage will need to be relatively high even for large fleets, when estimating mortality of dolphins with sparse and heterogeneous data. For example, with kill data as sparse and variable as was the case for common dolphins in 1987, nearly 100% coverage would be required to generate CV's lower than 20% regardless of fleet size (Fig. 6). With more frequent and less variable kill data, such as for northern spotted dolphin in 1987, CV's lower than 20% can be achieved with 50% coverage of 10-boat fleets (Fig. 6). When fleet size drops to 5 boats, even this relatively well-behaved data set requires coverage at about 75% to achieve CV's less than about 20%. With coverage as low as 25%, even a fleet size of 20 boats was insufficient to meet the management objective of 20% CV.

Fleet size

In smaller fleets, each data point comprises a larger fraction of the available mortality data. In particular, the influence of unusually large mortalities (e.g., the

[5]The possibility of high kills is more important than the possibility of low kills; this is because all dolphin group types experience zero kill frequently (therefore it is not unusual or unexpected). Also, zero kill is a definitive lower bound, while the upper bound on kill is limited only by the potential school size of the dolphin group.

one trip responsible for over 500 deaths of common dolphins) will be much greater in smaller fleets.

The accuracy and precision of mortality estimates are affected less by fleet size *per se* than by observer coverage level for a given fleet size, and the fleet's variability in kill per day. Kill per day is affected not only by the fishermen's choices of fishing methods and areas, but also by the type of dolphin found associated with a given school of tuna. Because these are not factors that can be controlled, small fleets will generally require higher coverage level than larger fleets to achieve a given level of accuracy and precision in mortality estimates.

Simulation procedures and estimates

The simulation procedure used in this study was designed to reflect the sampling process as it would occur in the real world. More precise and less biased estimates of mortality rates for the population of trips contained in the 1987 data set would have resulted from simple random sampling of the 124 trips in the data set as a whole. But simple random sampling implicitly assumes that all vessels are equal in fishing ability. This is not the case, and it is likely that some fleets as a whole may have greater (e.g., those newer to purse-seining and therefore less experienced) or lesser (e.g,, the more experienced fleets) mortality rates than the average for the ETP purse-seine fleet overall. In addition, in the real world, not all trips made by all purse-seine vessels fishing in the ETP would be available for sampling. Only trips made by the vessels in a particular fleet would be available for sampling, and only those vessels actually observed would contribute data. If vessels (or more properly, the crew) differ in their ability to release dolphins unharmed (or not), then fleets with more (or fewer) "low kill" vessels will have lower (or higher) mortality rates than other fleets of comparable size. Although it would have been possible to estimate the number of trips that would have been made, on average, by a fleet of a given size, and to have then randomly sampled that many trips from the 1987 data base, the results would have been unrealistically precise. The cluster sampling resulting from the selection of trips only after selecting vessels adds variability in the estimates but is more realistic than simple random sampling. The sampling scheme used here is a single stage cluster sampling, for which a ratio estimator is the most appropriate choice of estimation procedure (Cochran, 1977).

Discussion

Although the kill-per-day estimator used in this simulation study is no longer used by NMFS because 100% observer coverage has made estimation unnecessary, the results of this study have general implications for current estimation procedures based on kill per set (e.g., Hall and Boyer, 1986) and for mortality estimation procedures in general where data quality may vary between stocks.

The uneven structure of the data set for common dolphins has unfortunate implications for deriving estimates of mortality for dolphin groups that are characterized by having such infrequent and widely variable kill per day. Specifically, estimates of mortality can vary widely depending on which trips happen to be chosen. In our simulation, we could resample the total population of vessels repeatedly, thus generating relatively unbiased, though individually variable, estimates of mortality. In the real world, only one sample (one set of mortality data per dolphin group type) will be collected per year. If this sample is collected under low percent coverage, it appears very likely that the data may be affected by undetectable sampling biases. This bias is more likely to underestimate than to overestimate mortality because sets with large kill are rare and likely to be underestimated, even though the mortality during such sets may be responsible for a disproportionately large percentage of the total kill.

The problem with missing the rare large-kill sets is that the kill in these sets can apparently be one or two orders of magnitude greater than the "usual" kill. For very abundant groups, missing a few large kills will miss only a small percentage of the total number of dolphins in the group; underestimating mortality could be relatively harmless. For less abundant groups, the large kills might represent a significant proportion of the existing stock. Underestimating this mortality could lead to seriously underestimating the impact of mortality due to fishing operations on these stocks.

In the case of the 1987 data set for dolphin kill by the U.S. fleet, coverage greater than 96% (the highest observed) would be required for all boats in order to generate mortality estimates for common dolphin with CV's less than 20%. Alternatively, if only the most abundant groups are considered (e.g., offshore spotted dolphin), CV's less than 20% could be achieved with only 50% coverage of fleets as small as 5 boats. There appears to be no unique solution that is optimal for all groups.

In addition, the poor quality of the data presented here for common dolphin in fact underestimates the true extent of the problem for this species. In actual practice, the species is managed as three separate stocks rather than as one combined stock as presented here. The data are thus extremely sparse for the individual stocks, and the problems with estimating mortality, given anything less than full observer coverage, are greatly exacerbated.

The results of these simulations, in particular the results for five-boat fleets, and common dolphins, strongly influenced subsequent regulations related to comparability criteria for import of tuna caught by non-U.S. fleets. These regulations now require that mortality data for common dolphins be stratified separately from all other stocks, and that non-U.S. fleets meet the U.S. requirement for 100% observer coverage[6].

Of the three factors discussed here (dolphin group type, percent coverage, and fleet size), only percent coverage can be controlled by the sampling program. Our results imply that providing maximum protection for all dolphin groups would require mandating coverage to achieve a desired level of statistical precision for the dolphin group type with the least statistically stable data.

Acknowledgments

The U.S. fleet simulation study was designed by Bruce Wahlen. Nancy Lo and Peter Perkins provided insightful advice about sampling procedures and statistical methods. We thank also the participants in the Workshop on Estimating Dolphin Mortality, and two anonymous reviewers, who provided many useful comments during the review process.

[6]U.S. regulations for acceptable levels of observer coverage for non-U.S. fleets have progressed from a requirement for 33% coverage of trips made by fleets with 10 or more vessels, 50% of trips on fleets of 5–9 vessels, and 100% of fleets with 1–4 vessels during 1989 and 1990 (Federal Register 12/19/89;Vol. 54 #242, p. 51918–51923), to 75% of all trips regardless of fleet size during 1991 and 1992 (10/18/90; 55 FR 42235 and 10/8/91; 56 FR 50672), to the current requirement for 100% coverage regardless of fleet size (Federal Register 1/8/92; Vol. 57, #8, p. 668).

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Method and precision in estimation of dolphin school size with vertical aerial photography

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Abstract.—Field and laboratory procedures were used to acquire dolphin school size estimates from vertical aerial photographs. Multiple photographs were taken of 48 separate schools during a 1989 eastern tropical Pacific (ETP) dolphin abundance survey. During a 12-week "counting period," three readers did independent counts of dolphins in the photographs. For each school, the best photograph imagery was selected and the mean of the three independent counts was used to estimate its "true" size. The coefficient of variation (CV) for school size estimates (between-reader precision) averaged 5.4% and ranged between 1.2% and 14.6%. Most (92%) of the schools were estimated with precision, resulting in a CV of less than 9.0%. Within-reader CV averaged 3.5% and ranged 1.4%–7.1%, indicating that readers were quite precise. To test if reader methods were constant during the counting period, temporal trends in estimates were tested by linear regression analyses and a repeated-counts experiment with repeated measures analysis of variance (RM-ANOVA). Regression analyses indicated no significant temporal trends or bias in the deviation of counts from the means. The RM-ANOVA showed a significant "reader with time" interaction which was attributed to the relatively high variability between readers in counts made at the start of the experiment. Results suggested that methods were constant and counts were precise after an initial "warm-up" counting session.

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Visual estimation of the number of dolphins in a school is difficult because dolphins dive and the entire school is rarely visible at the sea surface at one time. Shipboard observer estimates can be highly variable and they may be biased (Scott et al., 1985; Anganuzzi and Buckland, 1989). Consequently, estimates of dolphin population abundance derived from visual survey data may be biased, and resulting management decisions aimed at conserving these populations may be inappropriate.

The Southwest Fisheries Science Center (SWFSC) has been conducting surveys to monitor temporal trends in the abundance of eastern tropical Pacific (ETP) populations of the pantropical spotted dolphin (*Stenella attenuata*), spinner dolphin (*S. longirostris*), striped dolphin (*S. coeruleoalba*) and common dolphin (*Delphinus delphis*). These surveys are one part of a multifaceted effort to conserve ETP dolphins subjected to incidental mortality in the international purse-seine fishery for yellowfin tuna, *Thunnus albacares* (Perrin, 1975; Wade and Gerrodette, 1992). Starting in 1987, in response to the problem of potential bias in visual school-size estimates, annual ETP surveys were complemented with aerial photography of dolphin schools. This allowed shipboard-observer estimates to be compared with estimates (of the same schools) taken from counts of dolphins in the photographs.

School size estimates derived from large-format (126-mm) aerial photographs were validated during a 1979 study when five separate schools (size range: 161–396 dolphins) were photographed and then captured in a tuna purse-seine net (Scott et al., 1985). Results showed that dolphin counts from the photographs were not statistically different from counts tallied by hand as dolphins were being released from the net. This suggested that counts from aerial photographs approximated "true" school size.

This report details photographic and counting methods used to derive dolphin[1] school size estimates from large-format vertical aerial photographs. Many of the techniques originated with Scott et al. (1985). The techniques were modified to support the photography and laboratory efforts associated with large-scale ETP dolphin population surveys (i.e., annual surveys of 120 days; covering a 19-million-km^2 study area). After a survey in 1989, three readers did independent dolphin counts from approximately 200 dolphin school photographs (multiple photographs were taken for 48 separate schools) over 12 consecutive weeks. For each school, the best photograph imagery was selected (according to criteria described below) and the mean of the three independent counts was used to estimate its true size. The coefficient of variation (CV) was used to characterize the precision (between-

[1]For purposes of this paper, "dolphin" refers to dolphins as well as small toothed-whales that are included in the photograph sample.

and within-reader) associated with the school size estimates.

One concern was that during the 12-week "counting period," readers might be inconsistent in the application of criteria (cognitive or physical methods, or both) used for counting. If readers were inconsistent, then this might affect the accuracy and precision of the estimates. A two-step approach was taken to address this concern. First, temporal trends in the deviation of counts from the means (see Sokal and Rohlf, 1981, p. 50) were evaluated by linear regression. This approach assumed that the mean of three reader counts was close to the true school size and that the sample error was normally distributed. If individual reader counts were not normally distributed, then this might indicate reader bias or a change in criteria. Second, readers did repeated-counts (at four time points during the counting period) of a known sample of photographed schools. This was done to evaluate the consistency of individual readers (within-reader precision) and to test the hypothesis that dolphin counts (from the same photographs) done at the beginning, middle, and end of the counting period were independent of temporal effects. If the hypothesis proved true, then this would support the idea that counting criteria were constant throughout the counting period.

Materials and methods

At sea

Aerial photographs analyzed in this report were taken with Chicago Aerial Industries KA-62 aerial cameras mounted vertically on a Hughes 500-D helicopter. The helicopter was stationed aboard the National Oceanic and Atmospheric Administration (NOAA) survey ship *David Starr Jordan*. All cameras had forward-motion compensation (to minimize photo image blur from aircraft movement) and a 76.2-mm lens. Large-format (126-mm) Kodak Aerochrome MS 2448 color film was used. In order to minimize the behavioral response (scattering and deep diving) of the dolphin schools to the helicopter, photographs were taken at 244 m (800 ft) altitude. The scale of the photographs at this altitude was 1:3200, the sea surface area in a photograph frame was 366 m^2, and a 2-m dolphin measured 0.63 mm on the film. The camera cycle rate was programmed to expose for approximately 80% film image overlap, i.e., 80% of the area photographed in one frame was photographed again in the next successive frame. Successive exposed photograph frames for a school were recorded as a complete "photo-pass." To enhance the probability of photographing an entire school, multiple photo-passes (avg.=5) were made over a school.

In the laboratory

Counts Light tables equipped with dissection microscopes (0.7× to 7× variable objective and 10× wide-field oculars) were used to view and count the dolphin images during counting. Dolphins were counted by hand-tally while being plotted with a permanent marker on a clear acetate overlay. The marked overlay, when moved to the image overlap area of adjacent frames and aligned over dolphins that were previously plotted, made it easier to identify those dolphins not yet counted in the photo-pass. The photo-pass was the unit on which school size estimates were based (Scott et al, 1985); each pass was counted independently by three readers.

Criteria for selecting the "true" school size For each of the 48 photographed schools, the readers chose (by group consensus) the one photo-pass where the mean of the reader counts was the best estimate of true school size. This decision was based on the precision of the three replicate counts and the reader assigned "quality ratings" for the photo-pass. Ratings reflected how confident the reader was in the accuracy of the count. For each photo-pass, readers independently assigned quality ratings ranging from 1 to 4. A rating of "1" indicated that a photo-pass had "excellent" quality. A rating of "2" indicated that the count was "good" despite the presence of some questionable images (i.e., it was difficult to discern and count dolphins accurately when images were partially obscured by light-glare or when photographic resolution was reduced for deep swimming dolphins because of loss of light with sea depth). A photo-pass was rated "3" or "fair" when more questionable images were encountered, but readers still believed the count was a close approximation of true school size. A photo-pass rated "4" was deemed unusable for size estimation because the reader felt there were too many questionable images and the count was not a reliable estimate of true school size.

One source of between-reader variation in dolphin photograph counts was reader error, where dolphins were missed or counted twice. Variation also occurred because readers differed in their interpretations of whether questionable images were dolphins or merely background water turbulence in the photographs. Based on the premise that "precision leads to accuracy" (Sokal and Rohlf, 1981), for each photo-pass, the CV of the three independent counts was used to monitor the reliability of the school size estimate. For photo-passes where the CV of the counts exceeded 10%, the dolphin school was re-counted (independently) to see if the precision of the estimate could be improved. If the CV was above 15% after a second count, the photo-pass was excluded from the study because the counts

were considered too variable and little confidence was placed in the accuracy of the estimate.

Analytical methods The CV was plotted against the variables "school size" and "quality rating" to evaluate how different school sizes and photograph image qualities affected precision. The CV was also used to characterize the precision of individual readers in repeated-counts as described below.

The percent deviation (PD) is a measure of how distant (+ or –) a count is from the mean. Because this difference is expressed as a percentage of the mean, the PD is a consistent index of deviation relative to changes in the mean. For the PD, let x_{ij} be the ith reader's determination of the size of school j. The average determination (or mean) over three readers of the size of the jth school was given by

$$\bar{x}_j = \frac{1}{3}\sum_{i=1}^{3} x_{ij} \ . \qquad (1)$$

The PD of x_{ij} is expressed as

$$PD_{ij} = \frac{x_{ij} - \bar{x}_j}{\bar{x}_j} * 100 \ . \qquad (2)$$

PD values were plotted to evaluate whether individual reader counts were normally distributed or readers were biased (i.e., tended to count high or low relative to the mean). To test for temporal trends in individual reader counts, PD values were regressed against the variable "time." Time represented the chronological sequence, unique for each reader, in which the 48 schools were counted. Logistically, it was impractical for readers to follow the same sequence in working with the photo-passes.

For the repeated-counts experiment, a known sample of six photographed dolphin schools (henceforth referred to as the "experiment schools"), which varied in school size and image quality, were counted four times. Counts were done once at the start of the counting period, then again every 25 days during the period. Changes with time (temporal trends) due to the variables of "school size," "image quality," and "reader" were tested by using a repeated measures analysis of variance (RM-ANOVA) model from Winer (1971; p. 337).[2] Outlier counts were identified by using Shapiro-Wilk's test for normality at $\alpha = 0.05$ (Shapiro and Wilk, 1965). After log-transformation, data met F-test requirements of homoscedasticity according to Levene's test at $\alpha = 0.05$ (computer program BMDP7D used, Dixon et al. 1988). The RM-ANOVA was computed using the software program SuperANOVA (Abacus Concepts, 1990).

Results and discussion

Scott et al. (1985) reported that dolphin school size estimates derived from aerial photographs were accurate and more precise than visual estimates. They found the standard deviation of estimates (log-transformed) averaged 6% of school size for photographic estimates and 10%–30% of school size for visual estimates. The CV for estimates (untransformed) of the 11 schools used in their precision analysis averaged 8.4% (range: 3.7%–15.1%) indicating slightly less precision when compared with estimates presented here (avg. CV: 5.4%; range: 1.2%–14.6%; Table 1). The difference is explained, in part, by their statistical model, which accounted for variance due not only to independent repetitive counts (2 to 4 per photo-pass), but also due to camera types (126- and 229-mm formats) and multiple photo-passes (2 to 7) for a given school. In the present study, variability was minimized by use of one type of camera (126-mm format) and by including only counts of the single best photo-pass for a school.

School size estimates averaged 146 and ranged between 4 and 633 (Table 1). Most schools (92%) were estimated with precision that resulted in a CV of less than 9.0%, and precision varied little with school size (Fig. 1 and Table 2). Estimate precision tended to decrease with decreased quality of the dolphin school photographs (Fig. 2). PD values (listed in Table 1) plotted for individual readers appeared normally distributed, indicating no between-reader bias in counts of the dolphin images.

Repeated-count data are presented in Table 3. Outlier values for experiment school number III (Table 3) resulted from reader error when dolphins were missed as the marked acetate was moved from the dolphin low density area of the photo-pass (in this case, the beginning of the photo-pass) to the high density area. Alternatively, when dolphins in the high density area were counted first, the precision of the estimate was improved because the majority of dolphins in the school were plotted and counted at the onset; this made it easier to track individual dolphins on adjoining frames.

Within-reader CV for repeated counts averaged 3.5%. Reader 2, the most experienced reader, was most precise in repeated counts (avg. CV: 2.6%; range: 1.4–3.8%) followed by reader 3 (avg. CV: 3.4%, range: 1.5–5.1%), and Reader 1 (avg. CV: 4.7%, range: 2.5–7.1%). The RM-ANOVA showed significant differences between

[2]Model detailed in: Gilpatrick, J. W. Jr. (1992). Using vertical aerial photographs to estimate dolphin school sizes: precision and consistency. U.S. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Southwest Fish. Sci. Cent., P.O. Box 271, La Jolla, CA 92038. Admin. Rep. LJ-92-35, 20 p.

Table 1

Counts, mean school size, CV, average quality ratings, and PD values for schools photographed in the 1989 ETP dolphin population survey.

School no.	Species codes[1]	Reader counts[2]			Mean school size	CV (%)	Avg. quality rating[3]	Reader PD[4]		
		R1	R2	R3				R1	R2	R3
1	S.COE	37	35	39	37.0	5.4	1.3	0.0	–5.4	+5.4
2	S.ATT	250	227	221	232.7	6.6	2.7	+7.5	–2.4	–5.0
3	S.LON	153	139	147	146.3	4.8	1.0	+4.6	–5.0	+0.5
4	S.COE	57	54	56	55.7	2.7	2.3	+2.4	–2.9	+0.6
5	S.ATT/S.LON	124	127	130	127.0	2.4	2.3	–2.4	0.0	+2.4
6	S.ATT/S.LON	192	196	216	201.3	6.4	1.7	–4.6	–2.7	+7.3
7	S.ATT/S.LON	274	236	242	250.7	8.2	1.0	+9.3	–5.9	–3.5
8	S.COE	48	47	49	48.0	2.1	1.0	0.0	–2.1	+2.1
9	F.ATT	17	18	18	17.7	3.3	1.0	–3.8	+1.9	+1.9
10	S.ATT/S.LON	81	78	77	78.7	2.7	1.0	+2.9	–0.9	–2.1
11	S.ATT/S.LON	412	416	398	408.7	2.3	1.3	+0.8	+1.8	–2.6
12	S.ATT/S.LON	101	106	118	108.3	8.1	2.3	–6.8	–2.2	+8.9
13	S.LON	20	20	21	20.3	2.8	2.7	–1.6	–1.7	+3.3
14	S.ATT/S.LON	107	113	113	111.0	3.1	1.3	–3.6	+1.8	+1.8
15	S.ATT	118	124	109	117.0	6.5	1.3	+0.9	+5.9	–6.8
16	S.ATT/S.LON	618	607	675	633.3	5.8	2.0	–2.4	–4.2	+6.6
17	P.ELE	400	391	399	396.7	1.2	1.0	+0.8	–1.4	+0.6
18	S.COE	58	53	60	57.0	6.3	1.3	+1.8	–7.0	+5.3
19	S.COE	52	55	61	56.0	8.2	1.7	–7.1	–1.8	+8.9
20	D.DEL	317	323	326	322.0	1.4	1.0	–1.6	+0.3	+1.2
21	D.DEL	326	312	367	335.0	8.5	1.3	–2.7	–6.9	+9.6
22	S.COE	24	23	25	24.0	4.2	1.3	0.0	–4.2	+4.2
23	G.MAC	19	18	19	18.7	3.1	1.0	+1.8	–3.6	+1.8
24	S.COE	75	77	68	73.3	6.4	2.0	+2.3	+5.0	–7.3
25	S.COE	34	30	32	32.0	6.3	1.3	+6.3	–6.3	0.0
26	S.COE	166	171	181	172.7	4.4	1.7	–3.9	–0.9	+4.8
27	D.DEL	66	64	60	63.3	4.8	1.7	+4.2	+1.1	–5.3
28	S.ATT/S.LON	216	216	233	221.7	4.4	1.3	–2.6	–2.6	+5.1
29	S.ATT	25	24	23	24.0	4.2	1.0	+4.2	0.0	–4.2
30	G.GRI	56	64	55	58.3	8.5	1.3	–4.0	+9.7	–5.7
31	S.BRE	4	4	5	4.3	13.3	2.0	–7.7	–7.7	+15.4
32	S.LON	576	678	548	600.7	11.4	2.3	–4.1	+12.9	–8.8
33	S.ATT	38	50	49	45.7	14.6	2.7	–16.8	+9.5	+7.3
34	S.COE	40	39	40	39.7	1.5	1.0	+0.8	–1.7	+0.8
35	S.ATT	88	90	80	86.0	6.2	1.7	+2.3	+4.7	–6.7
36	S.LON	315	324	350	329.7	5.5	1.3	–4.5	–1.7	+6.2
37	S.COE	26	25	24	25.0	4.0	2.0	+4.0	0.0	–4.0
38	S.COE	150	147	156	151.0	3.0	2.0	–0.7	–2.7	+3.3
39	S.BRE	36	34	35	35.0	2.9	1.0	+2.9	–2.9	0.0
40	S.ATT/T.TRU	284	293	337	304.7	9.3	2.3	–6.8	–3.8	+10.6
41	G.GRI	20	19	18	19.0	5.3	1.0	+5.3	0.0	–5.3
42	S.COE	35	34	35	34.7	1.7	1.0	+1.0	–1.9	+1.0
43	S.COE	73	74	88	78.3	10.7	2.0	–6.8	–5.5	+12.3
44	S.COE	154	154	144	150.7	3.8	1.3	+2.2	+2.2	–4.4
45	D.DEL	450	526	474	483.3	8.0	1.3	–6.9	+8.8	–1.9
46	S.COE	80	87	92	86.3	7.0	1.7	–7.3	+0.8	+6.6
47	S.COE	23	24	23	23.3	2.5	1.3	–1.4	+2.9	–1.3
48	S.COE	39	39	37	38.3	3.0	2.0	+1.7	+1.7	–3.7
Average:		145.5	145.9	147.4	145.5	5.4	1.6			

[1]Species codes: S.COE = *Stenella coeruleoalba*; S.ATT = *S. attenuata*; S.LON = *S. longirostris*; F.ATT = *Feresa attenuata*; P.ELE = *Peponocephala electra*; G.MAC = *Globicephala macrorhynchus*; D.DEL = *Delphinus delphis*; G.GRI = *Grampus griseus*; S.BRE = *Steno bredanensis*; T.TRU = *Tursiops truncatus*. Multiple species codes indicate mixed–species schools.

[2]Reader codes: R1 = Reader 1; R2 = Reader 2; R3 = Reader 3.

[3]Obtained by averaging the three independent reader assigned quality ratings.

[4]PD = percent deviation of reader count from the mean.

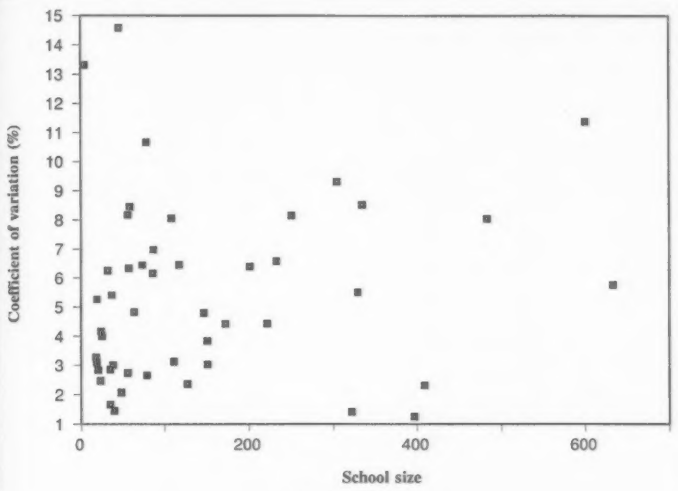

Figure 1

Comparison of mean school size and precision (using CV) of count estimates for schools (n=48) photographed during the 1989 ETP dolphin population survey.

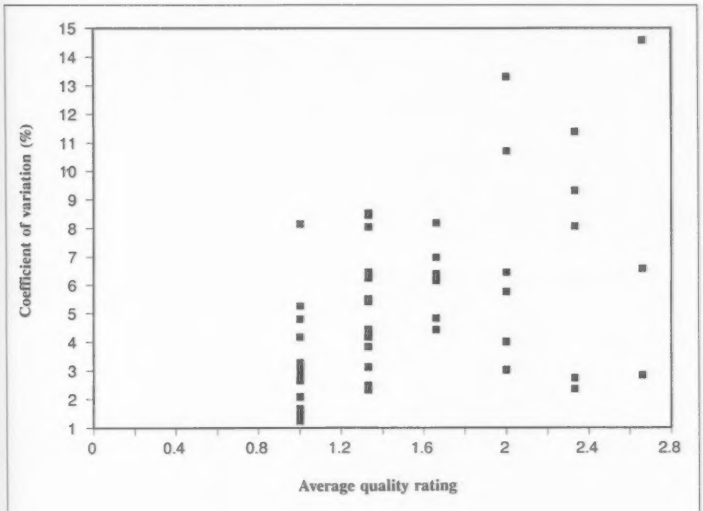

Figure 2

Relationship between photograph quality ratings (averaged for three independent readers) and precision (using CV) of count estimates for schools (n=48) photographed during the 1989 ETP dolphin population survey.

Table 2

Comparison of precision of estimates for small (<125 dolphins), medium (125–350 dolphins), and large (>350 dolphins) schools.

	CV (%)	
School size	Mean	Range
Small	5.3	(1.5–14.6)
Medium	5.2	(1.4–9.3)
Large	5.7	(1.2–11.4)
overall mean:	5.4	
overall range:		(1.2–14.6)

levels of the factors "school size" and "quality rating" (see between-factors; Table 4). This was expected because photographed schools of different sizes were intentionally selected for the analysis. Often, with RM-ANOVA, between-factor effects are confounded with different levels of the factors (as is true for the two factors above) and it is the temporal trends of the within-factor data that are of primary interest in the analysis (Winer, 1971, p. 299). There were no differences between readers in the overall means of their repeated counts (temporal trends not considered; P=0.7898; Table 4).

The F value for the within factor "Time" indicated no significant linear trend for repeated-counts (with the mean of the three reader counts). However, a significant interaction for "reader with time" (F=3.503; P=0.0258; df=6, 31) indicated there were differences between readers in the respective temporal trends of their repeated-counts (Fig. 3). To investigate the source of the significant effect, the trend data for each reader were tested *a posteriori* by contrasts among means with linear coefficients to weigh the log-transformed data (Sokal and Rohlf, 1981; Abacus Concepts, 1990). Results showed the mean of Reader 1's counts at time one differed significantly from the means of counts made at times two, three, and four (F=14.977; P=0.0005; df=1). This suggested that Reader 1's counts made at time one were important in causing the significant "reader with time" interaction.

Because the initial counts (at time one) of the experiment schools were done just prior to the start of the 12-week counting period, this suggested that

Table 3

Repeated counts experiment. Counts of the experiment schools were done at the start of the 12-week counting period, then repeated every 25 days during the period. Observations in parentheses are outlier values.

Schools	School size	Quality rating	Reader	Time			
				Time 1	Time 2	Time 3	Time 4
I	Small	Excellent	1	64	64	66	67
			2	69	66	68	70
			3	61	65	64	69
II	Small	Good	1	34	38	38	35
			2	36	35	36	36
			3	38	39	39	38
III	Medium	Excellent	1	(191)	242	230	236
			2	232	252	236	243
			3	(189)	(193)	(205)	(206)
IV	Medium	Good	1	(169)	214	194	203
			2	207	211	209	206
			3	213	195	194	205
V	Large	Excellent	1	401	468	468	450
			2	457	450	429	432
			3	440	451	429	467
VI	Large	Excellent	1	464	516	525	490
			2	541	527	534	496
			3	507	519	534	527

Table 4

Results from repeated measures analysis of variance (RM-ANOVA).

	df	Sum of squares	Mean square	*F*-value	*P*-value	G.-G.[1] *P*-value
School size	2	8.880	4.4400000	431.485	0.0001	
Quality rating	1	0.290	0.2900000	28.145	0.0003	
Reader	2	0.005	0.0020000	0.241	0.7898	
Error (between factors)	11	0.113	0.0100000			
Time	3	0.002	0.0010000	2.504	0.0775	0.0855
Time * school size	6	0.002	0.0003323	1.653	0.1661	0.2365
Time * quality rating	3	0.001	0.0003299	1.641	0.2001	0.1883
Time * reader	6	0.004	0.0010000	3.503	0.0092	0.0258
Error (within factors)	31	0.006	0.0002011			

[1]*P*-value corresponding to degrees of freedom adjustment using the Greenhouse-Geisser (1959) epsilon factor (=0.692) to correct for correlation of repeated measures. See Anderson (1958); Barcikowski and Robey (1984); Dunn and Clark (1987).

between-reader variance may have been atypically high early in the counting period as well. The regression analyses of PD values with time, however, did not provide strong evidence for this because reader counts were close to the mean values and no significant temporal trends were detected (Fig. 4). Probable cause for the significant effect was that readers were "out of practice" at the start, especially Reader 1. Prior to the

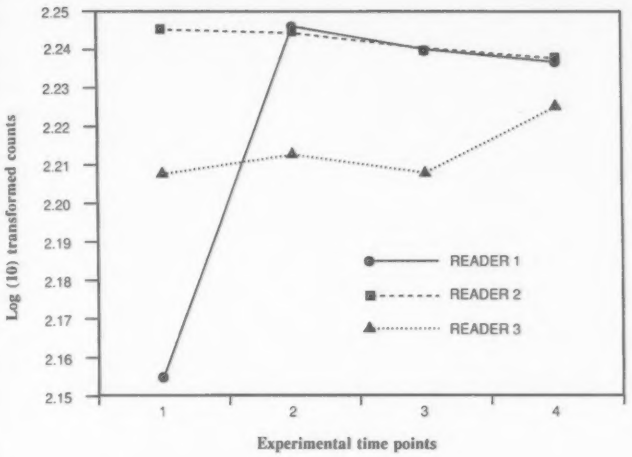

Figure 3

Temporal profiles of the means for counts (log-transformed) made by three independent readers during the repeated-counts experiment.

1991 counting period, a "warm-up" counting session was effective in improving estimate precision, i.e, between-reader CV went from an average of 8% to 4% within one day.

Conclusion

Results suggest that after a prolonged absence from interpreting dolphin school photographs and doing counts, a warm-up session, where readers are refreshed in counting technique, is important for minimizing reader error and improving precision. Estimate precision is also improved when plotting and counting is initiated at the dolphin high-density area in a photopass. The field and laboratory methods described in this report proved reliable in providing precise dolphin school-size estimates for population abundance studies. The CV statistic and PD index will be used for inter- and intra-annual quality control of dolphin school estimates taken from aerial photographs. The analytical methods used in this paper to describe precision and consistency are applicable to other fisheries and terrestrial wildlife research efforts where independent repetitive counts are conducted for parameter estimation.

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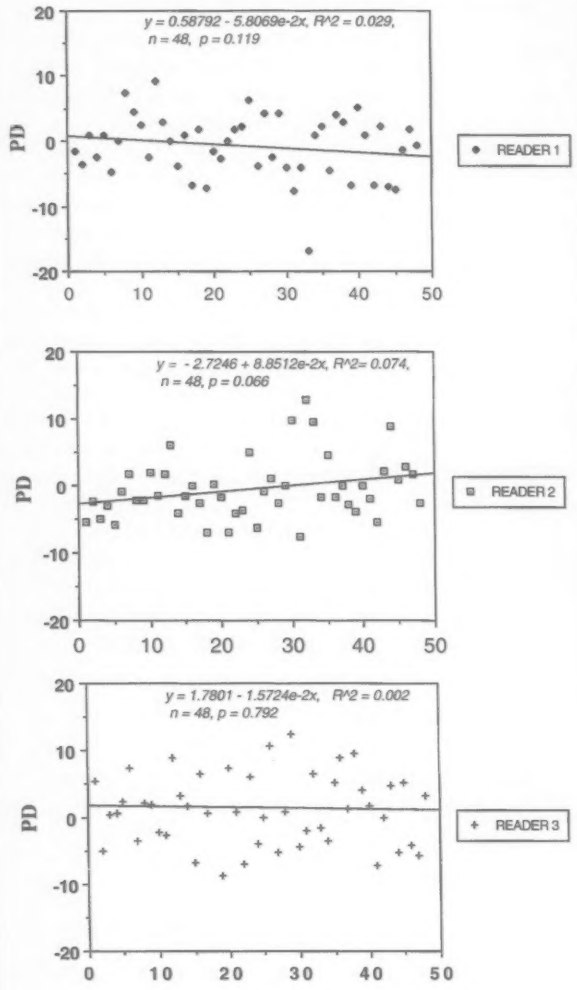
y = 0.58792 - 5.8069e-2x, R^2 = 0.029,
n = 48, p = 0.119
READER 1
y = - 2.7246 + 8.8512e-2x, R^2= 0.074,
n = 48, p = 0.066
READER 2
y = 1.7801 - 1.5724e-2x, R^2 = 0.002
n = 48, p = 0.792
READER 3
PD
Time (chronological sequence of photo counts)

Figure 4

Percent deviation (PD) values regressed on "time." Time represented the chronological sequence, unique for each reader, in which photographed schools were counted during the counting period. Zero on the y axis represents the mean count while negative and positive values represent the percent deviation of the count from the mean.

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Determination of age and growth of South Pacific albacore *(Thunnus alalunga)* using three methodologies

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Abstract.—Length-frequency data, vertebral-ring-counts, and length measurements of tagged fish were analyzed to independently estimate von Bertalanffy growth parameters for south Pacific albacore (*Thunnus alalunga*). As a working hypothesis, we assumed that the length-frequency modes and vertebral-rings are annual features. The MULTIFAN model fitted to length-frequency data provided estimates of L_∞ and K of 97.1 cm and 0.239 year^{-1}, respectively. Nine age classes were detected in the data. Growth varied seasonally: growth rate decreased to almost zero during August and peaked during February. Two to thirteen rings were visible in the vertebrae, and estimates of L_∞ and K of 121.0 cm and 0.134 year^{-1}, respectively, were obtained. No significant differences in growth parameters were detected between males and females. The growth rates predicted by the length-frequency and vertebral-ring-count models were very similar throughout the range of presumed age classes present in both data sets. These growth rates were consistent with length increment observations from 28 tag returns, which lends support to our assumptions of annual vertebral-rings and length-frequency modes.

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Fishery Bulletin 91: 649–663 (1993).

Albacore (*Thunnus alalunga*) have been fished in the south Pacific by Japanese longliners since 1952, and by Korean and Taiwanese longliners since 1958 and 1963, respectively (Wang, 1988). Japanese longliners ceased targeting albacore in the mid-1960's, but longline fishing for albacore by Korean and Taiwanese fleets has continued. Since 1960, annual longline catches have fluctuated between 25,000 metric tons (t) and 40,000 t (Anonymous, 1991). In the 1980's, a surface fishery for juvenile albacore developed, comprising drift gillnet vessels from Japan and Taiwan and troll vessels mainly from the United States, New Zealand, Fiji, and French Polynesia. By 1989, the annual surface-fishery catches of albacore had reached about 30,000 t (Anonymous, 1991). The rapid expansion of the surface fishery catalyzed concerns over potential overfishing and possible effects on longline catch rates of larger albacore. This in turn highlighted the need for detailed information on the fisheries and on the dynamics of the albacore population.

Investigations of albacore biology have been conducted since 1985 by scientists from the United States, France, New Zealand, and the South Pacific Commission. The goal of this research has been to obtain information on a range of biological and fishery-related processes, including growth, catch age structure, stock structure, and mortality. Albacore catch rates and size composition in the troll and drift gillnet fisheries have been monitored through a fishery observer program since 1988 (Hampton et al., 1989, 1991; Sharples et al., 1991; Labelle, 1993a). Albacore tagging programs were established in 1986 to provide information on movement, mortality, growth and approximate stock boundaries (Anon., 1986; Pianet et al., 1990; Labelle, 1993b). Catch sampling at major landing sites has been carried out to monitor catch size composition, to collect logbook data, and to obtain samples of bony structures and gonads for estimation of growth rates and spawning periodicity. Estimates of growth rates, and, in particular,

estimates of von Bertalanffy (VB) growth parameters, are important because they are necessary elements in the population dynamic models used in stock assessment.

We used three independent data sets to estimate albacore growth rates: length-frequencies, vertebral-ring-counts, and tag release-recapture statistics. Estimates of VB growth parameters were derived from the length-frequency and vertebral-ring-count data. In both cases, we assumed that the length-frequency modes represent year classes and the vertebral-rings represent annual features. This assumption was tested by using the length increment data from the tag returns.

Materials and methods

Collection of length-frequencies

Length-frequency data were collected by observers from seasonal surface fishery catches between December 1986 and May 1991. All albacore measured were caught by troll vessels on the principal fishing grounds for juvenile albacore, namely the Tasman Sea, along the central east coast of New Zealand, and near the Subtropical Convergence Zone (STCZ), mainly within the area bounded by 35–42°S and 130–170°W (Fig. 1). Throughout each fishing season (typically extending from December to April), observers sampled as many albacore as possible from the daily catches. Albacore were selected at random (i.e., unsystematically), placed on a graduated board, and measured from the tip of the snout (with the mouth closed) to the end of the median caudal ray. These fork-length measurements were rounded down to the nearest centimeter and recorded with the sampling date and fishing location. Tagged albacore were measured in an identical fashion and were combined with the length-frequency samples when it was appropriate.

Most of the length-frequency sampling occurred during 1988–1991 in the STCZ, where catch rates and fishing activity were greatest (Labelle, 1993a). In this region, over 100,000 albacore were measured; sufficient numbers were obtained to stratify samples by month for December–April each season. The number of measurements per month ranged from 351 to 18,908. These data were compiled into length-frequency histograms (Fig. 2), from which VB growth parameters were estimated by using the MULTIFAN computer program (Fournier et al., 1990).

Vertebra collection and processing

Caudal vertebrae were collected during the austral summers of 1986 and 1987 from albacore caught by research vessels trolling in the STCZ, the Tasman Sea, and over the Chatham Rise east of New Zealand (Fig. 1). Vertebrae were also collected during the austral winters of 1987, 1988, and 1989 from albacore caught by Japanese longline vessels fishing off the north east coast of New Zealand near East Cape

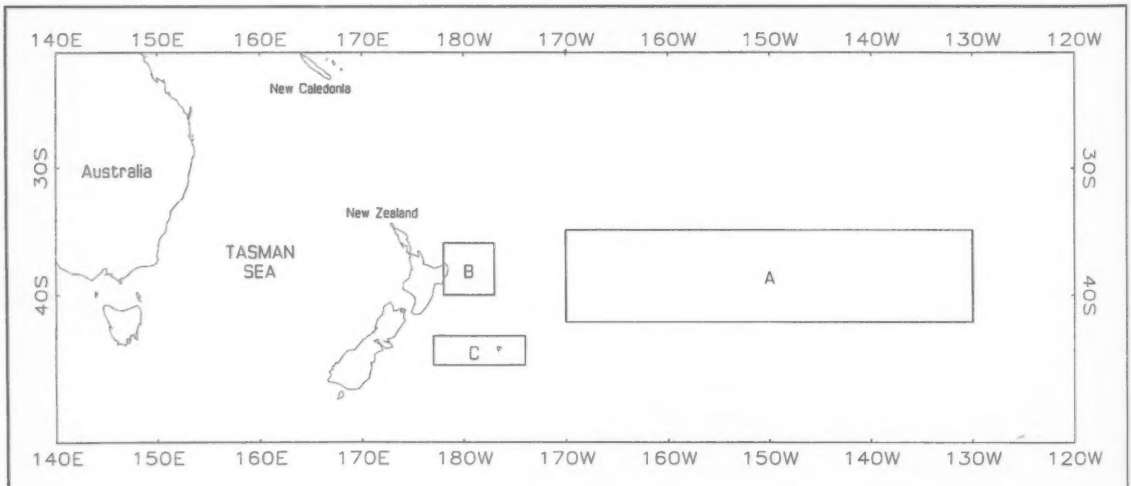


Figure 1

Sampling locations for the collection of length-frequency measurements and vertebrae. Region A covers the main troll fishing grounds centered over the STCZ. Region B delineates the longline fishing area adjacent to East Cape. Region C delineates the Chatham Ridge area where troll fishing occurs.

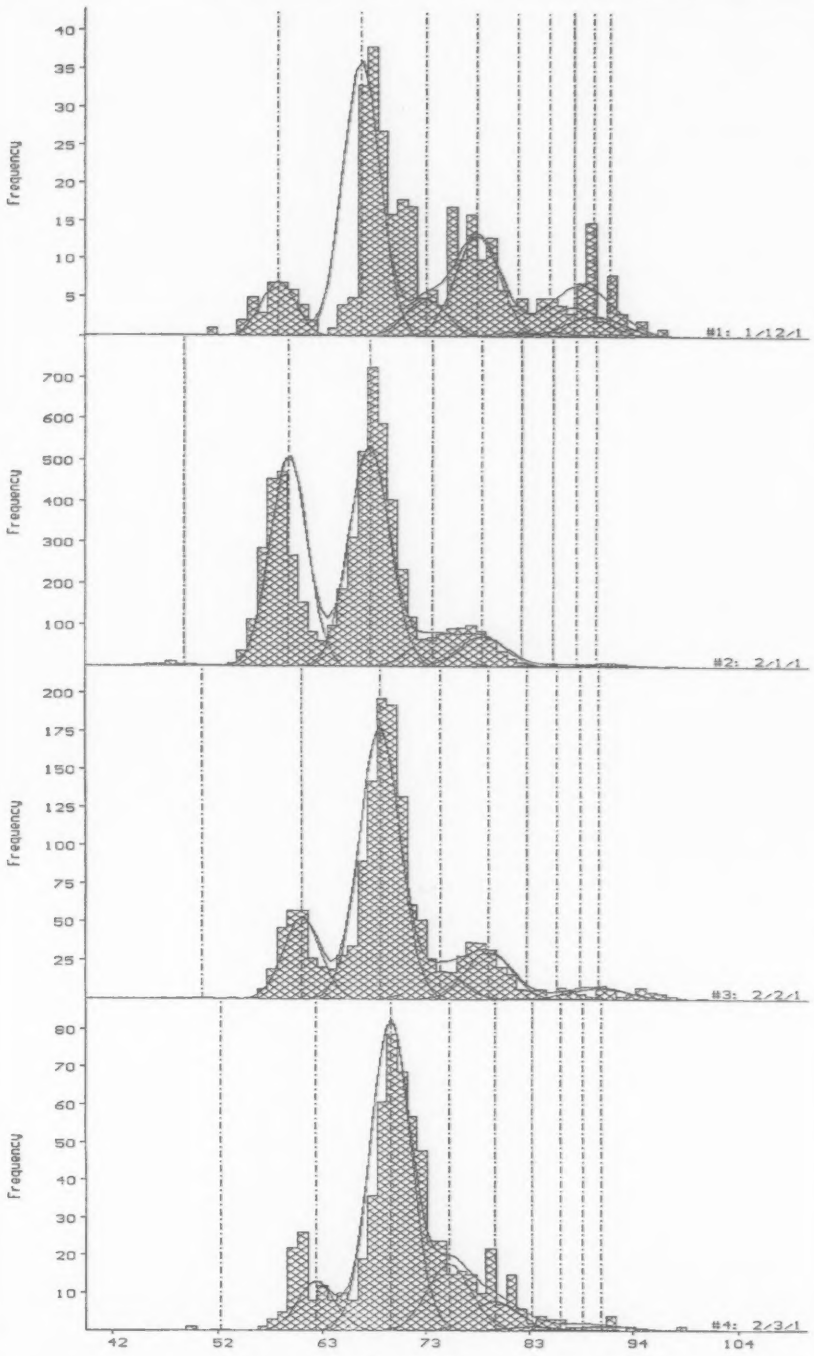
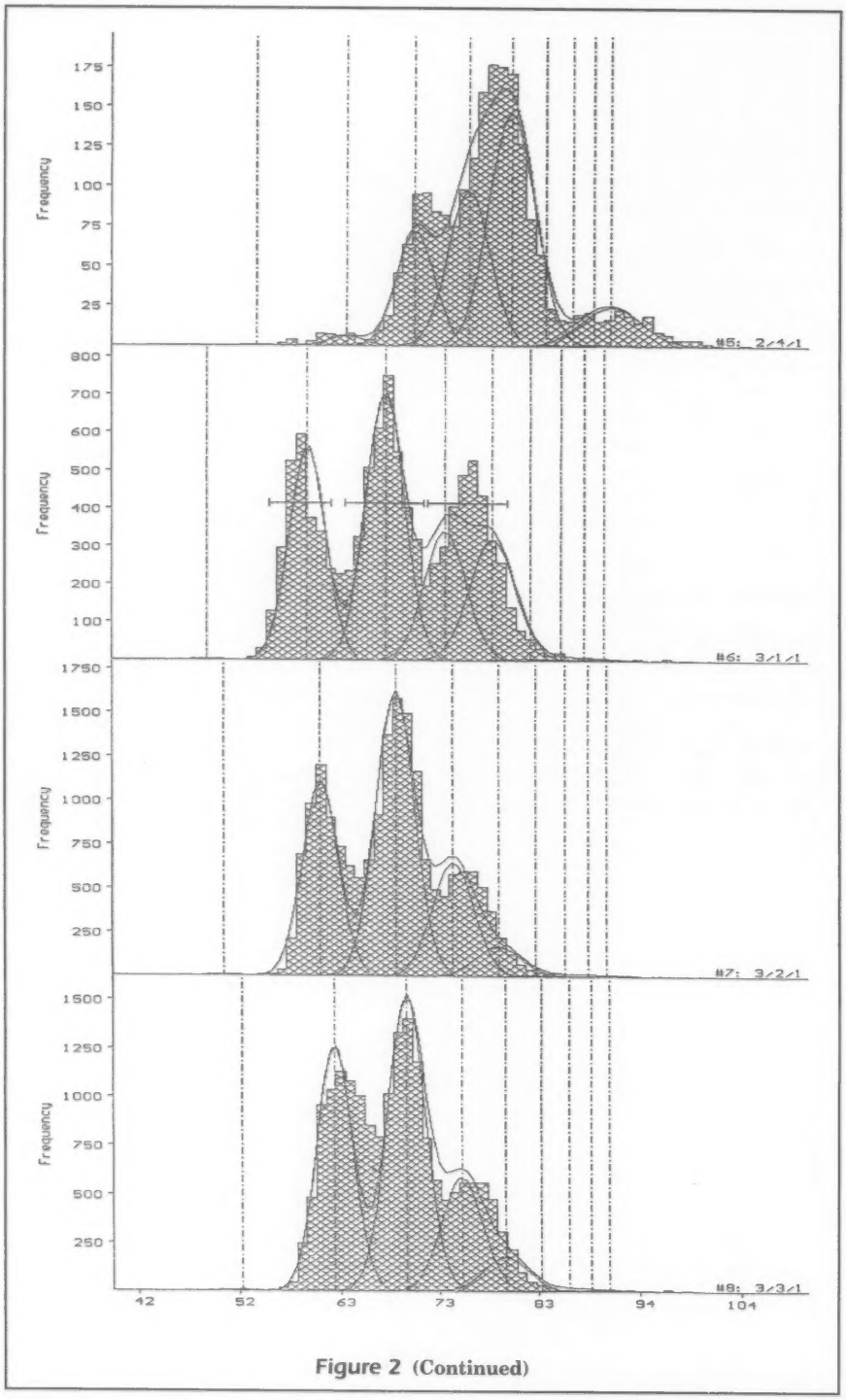


Figure 2

The south Pacific albacore length-frequency data set. The corresponding sampling period are given in the lower right of each histogram. The sampling data format is year/month/week; year 1 was 1988. No sampling was conducted during December 1989. The horizontal bars over the first three modes of sample no. 6 correspond to the user supplied bounds on the mean length of these age classes. The dashed vertical lines represent the estimated mean lengths-at-age in each sample. The inner smooth curves are the estimated numbers at age and the outer smooth curve is the estimated frequency distribution.



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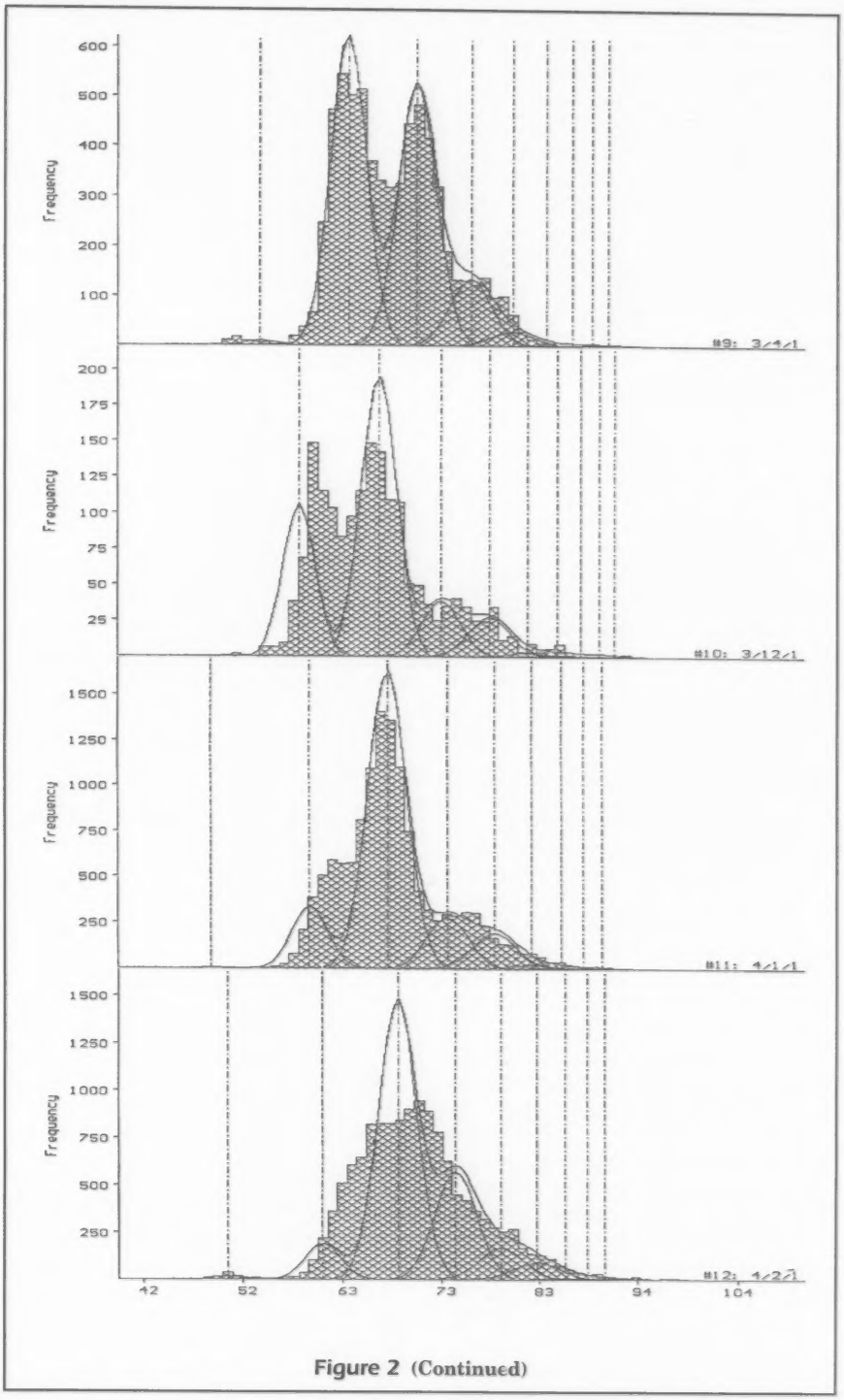

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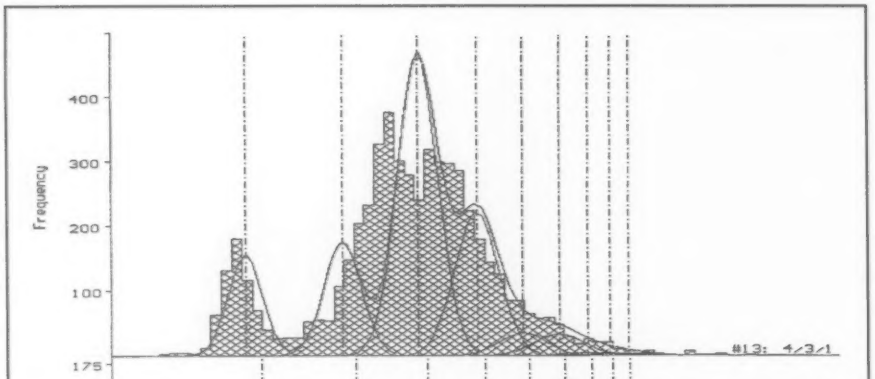

Figure 2 (Continued)

(Fig. 1). Altogether, 494 vertebrae were collected, of which 66% were from troll-caught albacore. All albacore sampled were first measured according to the procedure described previously. Vertebrae were removed by severing the caudal peduncle immediately anterior to the large bony keels. Vertebrae in the caudal peduncle were selected because they were easy to collect and their removal did not affect the market value of the fish. The caudal fins were trimmed, and the entire peduncle was placed in a plastic bag and frozen. Sex was recorded as often as possible (128 cases), but logistic difficulties prevented internal examination of the majority of the albacore sampled.

All vertebrae were processed in the laboratory according to the methods described by Berry et al. (1977), Berry (1978), and Lee et al. (1983). Caudal peduncles were thawed and the flesh trimmed from around the vertebrae. The 35th and 36th vertebrae were separated from the others with a knife and a fine-toothed hand saw. Each vertebra was then clamped in a vise by the keel, and sawed in half along the sagittal (dorsoventral) plane. Remnants of the cone jelly were removed and the anterior portion of the cone was lightly scrubbed.

The left lateral section was normally used for staining unless it had been damaged by cutting. Sections were soaked for 5–20 minutes in Alizarin red-S stain with Berry's (1978) "school tuna" solution, and then rinsed in fresh water. Sections stained too darkly were soaked in a 5% hydrogen peroxide solution until the stain faded sufficiently. Stained vertebrae examined under high intensity visible light displayed distinct band couplets on the anterior cone (see Johnson, 1983). Within each couplet, the bands are uniformly spaced around the cone and separated by a thin translucent zone. Each couplet is separated from adjacent ones by a wide opaque zone that becomes somewhat depressed towards the outside edge of the cone. Each couplet of twin bands was considered to be a distinct growth ring. The vertebral-ring-count was given by the number of complete couplet bands observed.

The investigators examined 20 stained vertebrae to derive criteria to be used in interpreting the ring structures. Vertebrae were then processed by the team: one biologist read 20 vertebrae while the other recorded data, and vice versa. At least 25% of the samples processed each day were examined by both persons to check consistency. Identical counts were obtained in

>95% of the cross-checks; samples were rejected if the discrepancies in counts could not be resolved. A linear relationship (r^2=0.94) was detected between ring counts for the 35th and 36th vertebrae of the first 200 albacore processed, and no significant difference in age composition was found between counts from the two sets of vertebrae (χ^2 test, P=0.9). Even though the 35th and 36th vertebrae were both suitable for study, we chose the 35th vertebrae because it was larger and easier to read in most cases.

Tagging operations

For tagging, albacore were caught mainly with commercial troll gear (Dotson, 1980). The tagging procedure was similar to that described by Laurs et al. (1976). Immediately after a strike, the fishing line was retrieved manually. Once the specimen was on board, the hook was removed and the albacore was quickly inspected for injuries to the gills, eyes, mouth, and palate. Albacore that were vigorous and not visibly injured were placed on a measuring board or tagging cradle (Kearney and Gillett, 1982) and were tagged as rapidly as possible with a stainless steel tube applicator that contained a 13-cm-long serially numbered spaghetti-type plastic tag with a single barbed nylon head. The tag was inserted at an oblique angle so that the barb was anchored among the pterygiophores of the second dorsal fin. Fork length was measured and recorded on audio tape, along with the date, time, albacore condition, and tag number. Albacore were returned to the water head first immediately after tagging.

Labelle (1993b) described the albacore tagging operations conducted in the south Pacific, and summarized the corresponding release-recapture statistics. Approximately 17,000 albacore were tagged and released from troll fishing vessels during 1986–92; the season totals ranged from 815 to 6,524. As of November 1992, recaptures of 42 tagged albacore had been reported; we obtained complete information on sizes and dates of release and recapture for 27 of 42 albacore. Most of these 27 tags were returned by fishermen, although some were detected during catch sampling at canneries and landing sites. Recreational fishermen also tagged and released an additional 3,646 albacore along the south east coast of Australia during 1973–92 (Matthews and Deguara, 1992). As of November 1992, 14 of these had been recovered but only one tag recovery record was sufficiently complete to allow inclusion of the data.

Data analysis

Length-frequency analysis The MULTIFAN computer program was used to estimate VB growth parameters from the length-frequency data under the assumption that the modes in the data represent year classes. A detailed description of the model is given by Fournier et al. (1990) and the program is described in the MULTIFAN 3 *User's Guide and Reference Manual* (Otter Research, 1991). MULTIFAN can incorporate specific structural hypotheses into models being fitted to the length-frequency data. The simplest structural hypothesis assumes that the mean lengths-at-age lie on a VB growth curve and that the standard deviations of length-at-age are identical for all cohorts. More complex structural hypotheses can be tested to determine if they provide a statistically significant improvement in fit to the data. The more complex hypotheses tested assume that the following processes can occur in the population sampled:

1 Sampling bias for the first cohort. This could result from selectivity during the sampling process induced by the fishing gear or the sampling method. Size selectivity was assumed to apply only to the first cohort and to decrease linearly with age until fish reach the second cohort.
2 Age-dependent standard deviation in length-at-age. For some fish populations, variation in length-at-age is not constant across cohorts. This hypothesis allows the standard deviation of length-at-age to increase or decrease linearly with age.
3 Seasonally oscillating growth. Seasonal growth patterns are known to occur in some fish populations (Pauly and Gaschütz, 1979). This process was incorporated into the growth model by adding two parameters, one representing the magnitude of the seasonal effect and the other determining the time of the year at which growth is slowest because of the seasonal effect.

We systematically fitted models incorporating all possible combinations of the above structural hypotheses and used likelihood ratio tests to identify the most parsimonious model structure. Fitting and testing procedures were done automatically by MULTIFAN, although some user-specified input was needed to ensure that the model exhibited stable behavior (see Fournier et al., 1990, for explanations).

Estimates of the VB growth parameters K and L_∞ were obtained along with parameters for sampling bias, age-specific standard deviations, and seasonal growth. In the absence of information on the age of the first age-class, MULTIFAN assumes that the VB curve passes through the origin (i.e., t_0=0). Estimates of age-at-length are based on this assumption.

Analysis of vertebrae data The VB growth parameters were estimated from vertebral-ring-count data and length data, under the assumption that ring counts indicate total age (in years). We did not attempt to

estimate fractional age based on an assumed birth date; therefore, all ages are integers. Maximum-likelihood estimates (MLE) were obtained according to the procedure described by Kimura (1980). The objective function (Φ) minimized to obtain the parameter estimates was the negative log-likelihood of the VB model:

$$\Phi = \frac{N \log_e (2\pi\sigma^2)}{2} + \frac{\sum_{i=1}^{N} \{l_i - [L_\infty (1 - e^{-K(t_i - t_0)})]\}^2}{2\sigma^2} \quad (1)$$

where: l_i = fork length of individual i,
t_i = presumed age of individual i,
N = number of individual measurements,
L_∞ = estimated asymptotic fork length,
K = estimated growth coefficient,
t_0 = estimated hypothetical age at zero length,
σ^2 = estimated variance associated with the length-at-age.

The objective function was minimized by using the Quasi-Newton algorithm in the NONLIN statistical module of the SYSTAT microcomputer program (Wilkinson, 1989). Asymptotic standard errors and parameter correlations were obtained from the Hessian matrix once the iteration process was complete (see Wilkinson, 1989). As suggested by Kimura (1980), the initial values supplied for the parameters were obtained from the Walford plot (Ricker, 1975).

Comparison of growth curves with tag-return data

We used the growth curves fitted to the two data sets and the observed length increments from the available tag-return data to test our assumption that the length-frequency modes represent year classes and vertebral-rings represent annual features. It must be acknowledged that there is no statistically correct means of comparing the models and the length increment data, because the growth curves derived from length frequencies and vertebral-ring-counts are based on age-length data. The only statistically correct predictions possible from these growth models are predictions of length (the dependent variable) from age (the independent variable). However, the length increment data can be related to the growth curves by first generating predictions from the fitted growth models which are then compared to the length increment data. This was accomplished by predicting lengths-at-recapture by using the standard Fabens (1965) length increment model:

$$l_{r_i} = l_i + (L_\infty - l_i)\left(1 - e^{-K\Delta t_i}\right) \quad (2)$$

where: l_i = length at release of individual i,
Δ_{ti} = time at liberty of individual i,
l_{r_i} = estimated length-at-recapture of individual i,

and L_∞ and K are the VB parameters estimated from the length-frequency or vertebral-ring-count data. As noted above, this procedure is not correct in the strict statistical sense because the growth models are not used to predict length from age (see Francis, 1988). However, for comparative purposes, the second method should reveal any gross departures from the "annual features" assumption applied in fitting the growth models. Some justification for this is presented later.

Results

Growth analysis based on length-frequencies

The most parsimonious model structure for the albacore length-frequency data set included seasonal growth and age-dependent standard deviation in length-at-age. The incorporation of first length bias did not significantly improve the fit. Parameter estimates and estimates of the means and standard deviations of length-at-age are given in Table 1. Note that we use the term "relative age class" to denote that estimates of absolute age are based on the assumptions that the length modes represent annual cohorts and t_0=0.

The seasonal growth phase estimate of 0.216 indicates that fastest growth occurs in February, and slowest growth occurs six months later in August at the end of the austral winter. The seasonal growth amplitude estimate (0.949) is near the upper limit of 1.0, indicating that growth is almost non-existent at that time of the year. Standard deviation in length-at-age increased progressively with age for the nine significant age classes detected.

The predicted aggregate length-frequency distributions fitted the observed distributions very well over the entire range of sizes (Fig. 2), and the predicted modes closely matched the actual modes in most months. The predicted modal distribution pattern indicates that there were usually four prominent age classes in troll catch samples.

Growth analysis based on vertebral-ring counts

The fork lengths of the albacore sampled ranged from 44 to 110 cm (Table 2), which corresponds to the size range of albacore caught in the surface and longline

Table 1
Estimated parameters from the MULTIFAN analysis, with predicted fork lengths (FL) and standard deviations (SD) of lengths-at-age.

Parameter	Estimated value	Relative age class	Predicted FL ($\bar{x}$, cm)	Length-at-age SD
von Bertalanffy K (cm·yr^{-1})	0.239	3.04	48.86	1.74
von Bertalanffy L_∞	97.100	4.04	59.12	2.03
Brody's rho (ρ)	0.788	5.04	67.19	2.29
Age of first age class (yr)	3.040	6.04	73.55	2.53
Mean length sampling bias	0.000	7.04	78.56	2.72
Seasonal growth phase	0.216	8.04	82.50	2.89
Seasonal growth amplitude	0.949	9.04	85.60	3.03
Mean SD	2.373	10.04	88.05	3.14
Ratio of first to last SD	1.861	11.04	89.98	3.24

Table 2
Summary statistics on sizes of albacore aged from vertebral-ring-counts.

Ring count	Sample size	Range (FL, cm)	Mean FL	Length-at-age SD	Coefficient of variation	Mean size increment
1	7	45–51	47.2	2.21	4.68	—
2	79	44–65	50.1	4.23	8.38	2.9
3	71	46–72	57.1	6.86	12.03	7.0
4	68	48–78	65.9	6.63	10.01	8.8
5	61	57–95	73.6	8.01	10.88	7.7
6	53	58–99	79.6	8.74	10.98	6.0
7	54	70–105	85.1	8.42	9.89	5.5
8	26	73–102	88.9	7.34	8.25	3.8
9	20	80–101	95.0	4.58	4.81	6.1
10	32	88–110	96.6	4.61	4.77	1.6
11	20	92–107	97.6	3.69	3.78	1.0
12	2	103–108	105.5	3.53	3.35	7.9
13	1	107–107	107.0	—	—	1.5

fisheries (Labelle, 1993a). Up to 13 rings, assumed to be annuli, were visible in the vertebrae sampled. Sample sizes for all relative age classes were greater than 20, except for classes 1, 12, and 13. Significant differences in the standard deviation of length-at-age were detected among the age classes (Bartlett's test, χ^2=64.683, P<0.001), and the standard deviation was greatest for relative age classes 5–7. This trend differs from the results obtained in the length-frequency analysis, which indicated increasing standard deviation with age (although only linear trends were possible in this analysis).

Differences in mean size between the successive age classes were calculated to reveal other anomalies in growth patterns. In theory, absolute growth should be rapid at first and decrease progressively in later life (see Ricker, 1975). The pattern observed for albacore agrees with these theoretical expectations, except for the high growth rates observed between relative ages 8→9 and 11→12 (Table 2). No statistical or biological criteria justified the omission of the data associated with ages 8→9 from the analysis. However, the large growth increment obtained for age 11→12 could simply be biased because of the small sample size (2) for relative age class 12.

The VB model was fitted to the aggregate vertebral-ring-count data for relative age classes 2–11. The data for relative age classes 1, 12, and 13 were excluded because of their small sample sizes. The VB model provided a good fit to the remaining data (Fig. 3). The residuals were generally well centered around zero and the slope of a linear regression of residuals against relative age was not significantly different from zero (F-test=0.001; P=0.973).

Of the albacore sexed, 59 were females and 70 were males. As these were distributed in a similar fashion with respect to relative age class as the pooled data set (females, males, and unsexed), the VB parameters were estimated for each sex (Table 3). Likelihood ratio tests (Kimura, 1980) were used to test for differences in growth parameters between sexes. No significant differences were detected for any of the tests carried out (Table 4). Therefore, the growth parameters estimated from the pooled data set were considered to be representative of growth patterns of male and female albacore within the size range sampled.

Comparison of growth curves

A direct comparison of the VB growth curves estimated from length-frequencies and vertebral-ring-counts is slightly complicated by the assumption of t_0 = 0 for the length-frequency analysis and the estimation of t_0 = –1.922 year for the vertebral-ring model. This inconsistency would result in the growth curves being out of phase by almost two years, apart from any other differences that might be present. One way of comparing the two curves is to compare the expected growth of

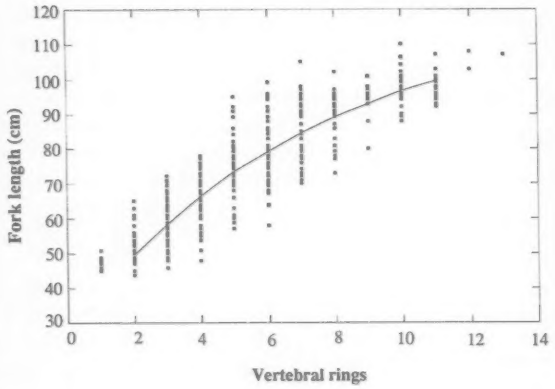

Figure 3

Observed albacore fork lengths (cm) for each ring-count category (relative age class). The von Bertalanffy curve fitted to data for relative age classes 2–11 is represented by the solid line.

an albacore of a specific initial size over time. This is equivalent to using the L_∞ and K estimates in a Fabens-type length increment model to make an approximate comparison of predicted growth trends independent of assumed or estimated t_0. Despite substantial differences in the L_∞ and K estimates from the two analyses, there was little difference in the predicted growth of a 40-cm albacore over time within the range of the data (Fig. 4).

Growth rates of tagged albacore

The 28 tag release-recapture records with complete growth data included a wide variety of sizes (61.0–96.5 cm), times-at-large (54–1,790 days) and release-recapture locations well distributed throughout the principle fishing areas. Estimated average growth rates during the periods at large were 0.17–1.13 cm·month^{-1}. For albacore at liberty <500 days, growth rate showed the expected decline with increasing mid-size (half way between release and recapture sizes); but this decline was negligible for albacore at liberty >500 days (Fig. 5). Given the relatively small number of records available, no attempt was made to estimate the VB growth parameters based on tag release-recapture data.

Comparison of tag return growth increments with the fitted models

To compare the consistency of the VB models derived earlier with the tag-return data, we first calculated sets of predicted recapture lengths for the 28 tag return records by using the two sets of L_∞ and K estimates, derived from length-frequency and vertebral-ring-count data, in Equation 2. We also made a third set of predictions using the L_∞ estimate and twice the K estimate obtained from the length-frequency analysis (L_∞=97.1 cm, K=0.478 yr^{-1}). These parameters are consistent with an assumption that the observed

Table 3

MULTIFAN parameter estimates of the VB growth model based on the vertebral-ring-counts. Parameter estimates are based on all albacore of relative ages 2–11 (top); females only of ages 2–9 (middle); and males only of ages 2–10 (bottom). Lower and upper bounds are the upper and lower limits of the 95% confidence intervals. MLE=maximum-likelihood estimates.

Sample content	Parameters	MLE	SD	Lower bound	Upper bound	Correlation matrix		
						L_∞	K	t_0
All albacore	L_∞	121.024	5.193	110.846	131.202			
ages 2–11	K	0.134	0.016	0.104	0.165	–0.981		
n = 484	t_0	–1.922	0.291	–2.492	–1.350	–0.880	0.951	
	σ^2	46.102	2.964	40.293	51.910	0.004	–0.004	–0.004
Females	L_∞	169.320	54.532	62.440	276.200			
ages 2–9	K	0.077	0.045	–0.011	0.166	–0.994		
n = 58	t_0	–2.573	1.109	–4.745	–0.400	–0.900	0.940	
	σ^2	40.775	7.574	25.930	55.621	0.026	–0.026	–0.024
Males	L_∞	122.037	14.332	93.947	150.127			
ages 2–10	K	0.168	0.054	0.063	0.274	–0.980		
n = 70	t_0	–0.907	0.649	–2.179	0.365	–0.847	0.929	
	σ^2	64.262	10.862	42.972	85.551	0.002	–0.002	–0.002

Table 4

Likelihood ratio tests comparing von Bertalanffy parameter estimates for male (1) and female (2) albacore, based on the procedure described by Kimura (1980).

Hypothesis	Linear constraints	Parameter estimates						Residual SS	χ^2	df	P
		$L_{\infty 1}$	$L_{\infty 2}$	K_1	K_2	$t_{0,1}$	$t_{0,2}$				
1	none	122.037	169.320	0.168	0.077	−0.907	−2.573	6863.3			
2	$L_{\infty 1}=L_{\infty 2}$	129.504	129.504	0.145	0.134	−1.180	−1.561	6911.0	0.104	1	>0.70
3	$K_1=K_2$	131.088	127.932	0.140	0.140	−1.265	−1.455	6922.4	0.129	1	>0.60
4	$t_{0,1}=t_{0,2}$	131.197	128.411	0.138	0.141	−1.350	−1.350	6933.6	0.153	1	>0.50
5	Same L_∞, K, t_0	131.157	131.157	0.136	0.136	−1.385	−1.385	6948.1	0.184	3	>0.50

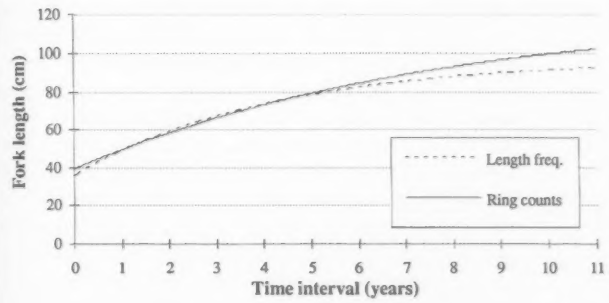

Figure 4

Growth of south Pacific albacore from an initial length of 40 cm as predicted by models fitted to length-frequency and vertebral-ring-count data.

length-frequency modes are semestral, rather than annual features. We then compared each set of predictions to the observed recapture lengths.

The growth models derived from length-frequency and vertebral-ring-count data, assuming that the modes and rings are annual features, appear to be consistent with the tag return length increment data (Fig. 6). Deviations of observed from predicted recapture lengths are both positive and negative and are generally within the range of expected individual variation. In contrast, the growth model based on the assumption of semestral length-frequency modes overestimates the recapture lengths. On this basis, the tag return length increment data lend support to our initial assumptions concerning the length-frequency modes and vertebral-rings.

Discussion

Estimates of the VB growth parameters for south Pacific albacore based on the analysis of length-frequencies and vertebral-ring-counts were generally within the ranges of values reported for albacore from other regions (Table 5). The only exception was the L_∞ estimate obtained with MULTIFAN, which was lower than all other estimates reported. This could be partly due to the fact that the data set used for the MULTIFAN analysis did not include length-frequency samples from longline catches, so the largest size classes were not well represented. Nevertheless, we do not attach any biological significance to this or to the differences in VB parameters estimated from the two data sets in this study. Despite differences in the parameters, the predicted growth patterns based on the two analyses are remarkably similar. This often noted feature of the VB growth model (e.g., Knight, 1968; Francis, 1988) highlights the potential pitfalls of comparing growth only on the basis of VB growth param-

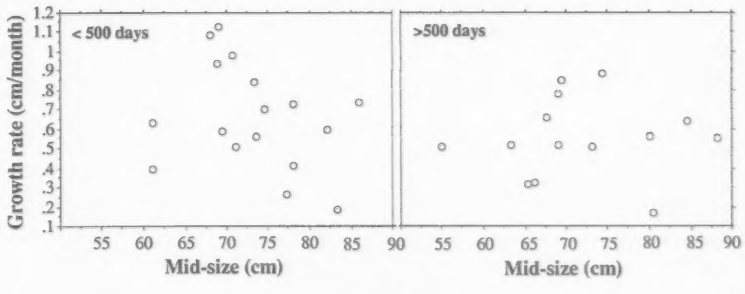

Figure 5

Individual growth rates of tagged south Pacific albacore for two time-at-liberty categories. Mid-size is the fork length half way between the release and recapture lengths.

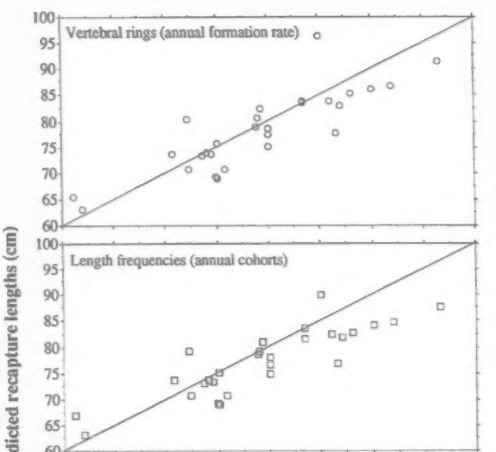
Vertebral rings (annual formation rate)
Length frequencies (annual cohorts)
Length frequencies (semestral cohorts)
Predicted recapture lengths (cm)
Observed recapture lengths (cm)

Figure 6

Predicted versus observed recapture lengths of tagged south Pacific albacore. The three panels depict recapture lengths predicted on the basis of models fitted to length-frequency data assuming annual cohorts, vertebral-ring-count data assuming an annual formation rate, and length-frequency data assuming semestral cohorts. The lines cover the points corresponding to a perfect match between predicted and observed lengths.

eters. In this study, the VB model was preferred to alternatives because it was used in available software such as MULTIFAN. There were no indications from the data that the VB model was not appropriate for modelling albacore growth, although a Gompertz model (Ricker, 1979) appeared to provide an equally good fit to the vertebral-ring-count data.

In fishes, rings are thought to form in bony tissues as a series of growth checks separated by zones, usually more opaque, that are associated with growth (Casselman, 1983). The usual inference that rings are formed yearly is based on the observation that growth rate, particularly for temperate species, is frequently seasonal as a result of its dependence on temperature. Growth checks are thought to occur during the period of the year when the water is coldest; high growth rates occur during periods of warmer water temperatures. Length-frequency analysis provided evidence that juvenile albacore growth is strongly seasonal; therefore this seasonality could be the basis of ring formation in vertebrae. However, the relationship of water temperature with this seasonality is unknown. Albacore, while being predominantly a temperate species, are also found in tropical waters, particularly as adults. In common with other *Thunnus* species, they occupy a three-dimensional habitat within wide temperature limits (14–20°C) and are capable of physiological thermoregulation, maintaining body temperatures of up to 15°C warmer than that of the surrounding waters (Morrison et al., 1978). It is possible that ring formation in the bony tissues of albacore is influenced by these biological characteristics and by more complex biological cycles, such as feeding and reproduction, as well as the temperature of the water that they occupy.

As a working hypothesis, the length-frequency modes and vertebral-rings were assumed to be formed each

Table 5

Reported estimates of the von Bertalanffy growth parameter albacore from various regions around the world.

Data source	Sampling region	Aging method	L_∞	K	t_0	Size range (FL, cm)
Clemens (1961)	N. Pacific	Tagging	135.6	0.170	–1.870	54–77
Shomura (1966)	N. Pacific	Tagging	118.8	0.250	1.999	60–91
Shomura (1966)	N. Pacific	Scales	114.4	0.308	0.818	65–120
Shomura (1966)	N. Pacific	Scales	145.3	0.159	–0.056	50–120
Laurs and Wetherall (1981)	N. Pacific	Tagging	125.0	0.199	—	47–92
Shomura (1966)	N. Pacific	Vertebrae	104.8	0.431	1.504	69–90
Shomura (1966)	N. Pacific	Scales	145.3	0.150	–0.396	40–95
Bell (1962)	N. Pacific	Scales	108.8	0.225	–2.273	51–94
Huang et al. (1991)	Indian	Scales	128.1	0.162	–0.897	64–106
Beardsley (1971)	Atlantic	Length data	140.0	0.141	–1.830	68–114
González-Garcés and Farina-Perez (1983)	N. Atlantic	Dorsal spine	140.1	0.129	–1.570	38–110
Santiago (1992)	N. Atlantic	Length-freq.	117.9	0.200	—	48–106

year in the growth models presented in this paper. Laurs et al. (1985) validated the daily increment formation rates in sagittae of tagged north Pacific albacore that had been injected with oxytetracycline (OTC) prior to release and concluded that the increment counts, adjusted upwards by 5%, provided an accurate estimate of absolute age. Preliminary estimates of absolute age of south Pacific albacore were obtained from counts of otolith increments, assuming similar increment deposition rates as for the north Pacific population (Wetherall et al., 1989). These results indicated that south Pacific albacore grew about twice as fast as our estimates suggest. This would suggest that the length-frequency modes and vertebral-rings analysed here are more likely to be semestral than annual features.

The length increment data from 28 tag returns presented in this study clearly favor the hypothesis of annual length-frequency modes and vertebral-rings over a semestral formation rate. Given the assumption that length-frequency modes represent cohorts of albacore spawned during the same season, we would expect the temporal spacing of length-frequency modes to correspond to the frequency of spawning seasons. Both gonadosomatic indices and microscopic examination of gonad tissue of south Pacific albacore clearly indicate a single annual peak in spawning activity during the austral summer (Ramon and Bailey, 1993).

Most of the evidence therefore points towards annual length-frequency modes and vertebral-rings, although the conflicting results obtained from "daily" growth increment counts on otoliths are deserving of further study. It would be particularly useful to estimate increment formation rates in vertebrae and otoliths of tagged south Pacific albacore injected with OTC before release, as was done for the north Pacific population. During 1986–1989, 3,341 tagged south Pacific albacore were injected with OTC, and to date, three have been recaptured and the relevant hard parts obtained. It is hoped that examination of this material will, in due course, clarify increment deposition rates in south Pacific albacore.

A previous review of the available information on stock structure lead Lewis (1990) to conclude that the south Pacific population probably had very limited exchange with those of the North Pacific and the Indian Ocean. Parasite studies (Jones, 1991), tagging programs, and ongoing electrophoretic surveys have not yet revealed the existence of separate sub-stocks within the south Pacific population. Thus, the growth rates reported here could be considered as representative of the growth patterns in the entire south Pacific albacore population. However, we cannot reject the hypothesis that populations from other oceans have significantly different growth patterns. It is also plausible that local phenomena, such as land mass effects and upwelling zones that can affect the growth rates of albacore within certain regions of the south Pacific.

Acknowledgments

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Reproductive biology of the false southern king crab (*Paralomis granulosa*, Lithodidae) in the Beagle Channel, Argentina

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Abstract.—Reproduction of the false southern king crab (*Paralomis granulosa*) in two localities of the Beagle Channel, Argentina, was studied by monthly trap sampling during 1989 and 1990. Size at gonadal maturity in males (50.2-mm cephalothoracic length, CL) and females (60.6-mm CL) was significantly less than size at morphometric maturity (57.0-mm CL in males; 66.5-mm CL in females). Embryonic development lasted 18–22 months. During this period, there was apparently a 10–12 month diapause. In one of the two localities, development of eggs in a given clutch was very heterogeneous, suggesting simultaneous occurrence of eggs with 12- and 22-month development periods. Larval hatching took place mainly during winter. Female *P. granulosa* molted during November and mated immediately after. Biennial reproduction was detected on the basis of ovarian and embryonic development, and on the basis of shell condition. Thus, two different female groups occur in the population of the Beagle Channel. Fecundity increases with size (1,441 to 8,110 eggs per female) and is significantly less at the end than at the start of embryogenesis. Ovaries and brood each represented at most 6–7% of body weight. *Paralomis granulosa* is the only representative of its genus that inhabits shallow water and apparently retains some reproductive features of its deep-water relatives.

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Crabs of the genus *Paralomis* are lithodids that inhabit the Atlantic, Indian, and Pacific Oceans, ranging in depth from 5 to 4152 m. The false southern king crab, or centollón in Spanish, *Paralomis granulosa* (Jacquinot, 1847), inhabits the Pacific Ocean from Paso Tenaún (Chile; 40°S) to Cape Horn, and the Atlantic Ocean from 56°S to the Golfo San Jorge (Argentina; 47°S) including the Islas Malvinas (Falkland Islands) at depths of up to 50 m (Macpherson, 1988).

Paralomis granulosa and the southern king crab (*Lithodes santolla*) constitute the main crustacean fisheries off the southern tip of South America. Commercial exploitation of *P. granulosa* started in the early 1970's. The largest catches were recorded in 1986 and amounted to 1,300 metric tons for Argentina and Chile. In Argentina, during the last three years, the yield of *P. granulosa* was at least twice as great as that of *L. santolla*. Although commercial fishing for *P. granulosa* began more than 15 years ago, studies on the life history of this species are few. Management studies were conducted in Chile which resulted in fishing regulations, such as a minimum legal size and gear restrictions (Campodónico et al., 1983). There is virtually no information on the life cycle of *P. granulosa* in South America, except for reports on larval development (Campodónico and Guzmán, 1981), larval ecophysiology (Vinuesa et al., 1989), and diets of juveniles and adults (Comoglio et al., 1990).

In this study we document aspects of the reproductive biology of *P. granulosa*, from data collected during monthly sampling in the Beagle Channel during 1989 and 1990. Data on the reproductive cycle, fecundity, embryogenesis, and other life history traits were examined to acquire basic information on the biology of this commercially valuable species.

Materials and methods

The fishing area was located along the Beagle Channel between Lapataia Bay (west) and the Moat Channel (east) (Fig. 1): fishing depths ranged from 10 to 60 m. The Beagle

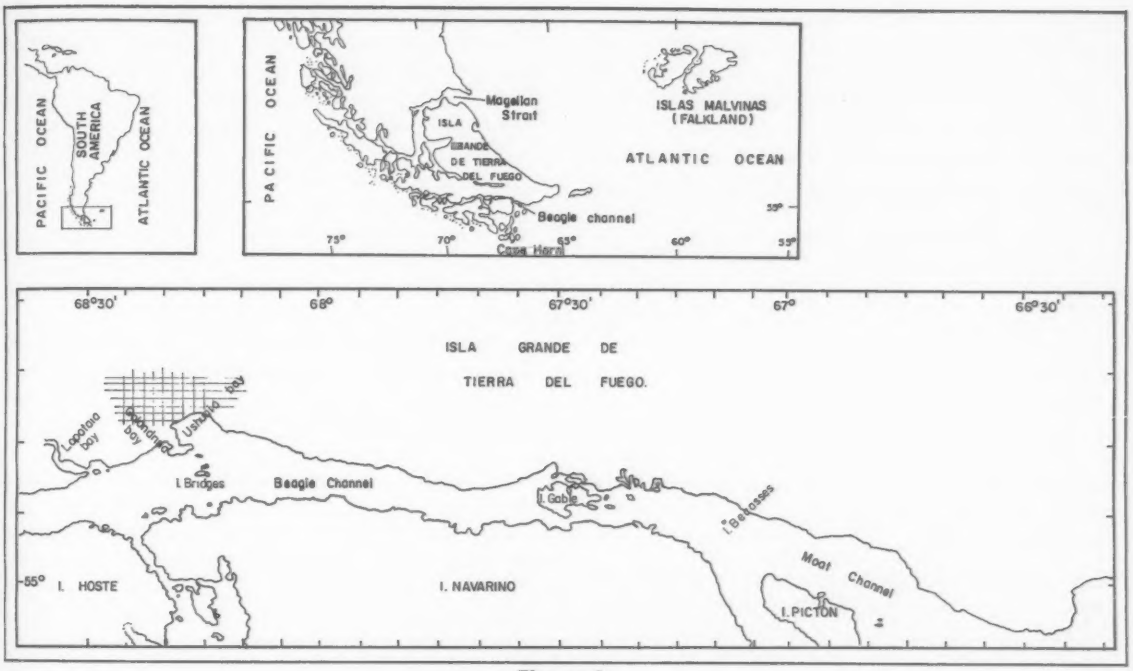

Figure 1
Location of sampling area.

Channel is an elongate glacial valley which stretches east-west, extending between the Isla Grande de Tierra del Fuego, and Navarino and Hoste Islands (Rabassa et al., 1986). It is 210 km long and on average 4 km wide and has a maximum depth of 160 m. Surface seawater temperatures range from 4.2 to 4.3°C in August and from 8.9 to 9.8°C in January; the annual mean temperature varies from 6.5 to 6.8°C. Salinity ranges from 26.7‰ in November–December to 31.3‰ during July and is highly variable during October and November because of ice melt.

Monthly samples were obtained from commercial catches from January 1989 to December 1990. Crabs were captured in traps deployed on bottom lines with 10 traps each. Traps were fished for 48 hours and usually baited with chicken, fish, horse, pig, cow, or lamb meat and bones. Each month 30–40 females and 10 males were randomly selected from the catch of a single line, placed in tanks with circulating seawater and transported alive to the laboratory. Carapace length (CL, midline distance between posterior orbital margin, excluding the rostral spine, and the posterior median margin) and right chela height (CH, measured at the anterior dorsal margin of the propodus, between the two anterior spines) of each crab were measured to the nearest 0.1 mm with a vernier caliper. Live body weight was measured to the nearest 0.01 g. The carapace of each crab was aged according to the following subjective scale:

1 Postmolt (POM): Shell soft, bright red, membranous and non-calcified, without epibiota.

2 Early Intermolt (EIM): Shell hard but brittle, bright red, without epibiota.

3 Median Intermolt (MIM): Shell hard, red to brown, variably covered with epibiota such as serpulids.

4 Advanced Intermolt (AIM): Shell hard, dark red, with numerous epibionts including balanids (6–8 mm maximum basal diameter), bivalves (*Mytilus edulis, Aulacomya ater*, 4–6 mm length), serpulids, and other calcareous polychaetes, bryozoans, and brown algae.

5 Premolt (PRM): Similar to AIM but the old shell is partially raised, revealing the new shell.

For both sexes, we used two methods to determine the size at which 50% of individuals were mature:

- Analysis of reproductive features. Females were considered to be mature if they had eggs or empty egg cases attached to the pleopods; males were considered to be mature if spermatophores were found in their vas deferens. The proportion of mature individuals was calculated for each 5-mm-CL interval and a logistic function was adjusted to provide 50% maturity.
- Allometric growth of the right chela. This method is based on the relative change in chela height at sexual

maturity (Hartnoll, 1978). We used MATURE1, the computer method described by Somerton (1980) to fit a pair of intersecting straight lines to plots of chela height on carapace length. The method determines the regression lines for crabs that are assumed to be immature and mature, based on minimum observed size at gonadal maturity and maximum size at gonadal immaturity, respectively. Then it extends the lines to the central area of the plot, where immature and mature crab are mixed, by iteratively assigning each point to either line, then recalculating lines, until no points switch lines on two successive iterations. On the basis of this classification, the proportion of mature individuals was calculated for CL-size intervals of 5 mm in males and of 4 mm in females and a logistic function was adjusted to provide 50% maturity (Wenner et al., 1974). Measurements of partially regenerated chelae were excluded from this analysis. Data for crabs <50 mm CL were obtained from a study of juvenile growth (Lovrich, 1991).

Fecundity was defined as the number of eggs per clutch. In the laboratory, pleopods with attached eggs were removed from each female and preserved in buffered 10% formalin in seawater. Later the eggs were detached from the pleopods and the clutch was blotted and weighed to the nearest 0.01 g (*WC*). Three subsamples were then weighed to the nearest 0.01 g (*ws*) and eggs in each subsample were counted (*ns*). Fecundity (*F*) was calculated as:

$$F = \sum_{i=1}^{3} [(WC * ns)/ws] / 3 .$$

Estimates of fecundity based on counts of three subsamples did not vary by more than 5%.

Egg characteristics, color, developmental stage, presence of chromatophores and appendage development were determined monthly with the aid of a stereoscopic microscope. Fifteen *P. granulosa* females were kept in a 600-L and 250-L tank in a controlled-environment room with photoperiod, temperature, and salinity adjusted to natural environmental variations from April 1990 to May 1991. Crabs were fed *ad libitum* with limpets, mussels, and fish 2–3 times a week. A subsample of 15–20 eggs was taken from each egg mass at weekly intervals at the beginning of the study and at monthly intervals thereafter. Eggs from females kept in the laboratory and eggs preserved in formalin were separated, and the maximum diameter of each egg was measured to the nearest 0.01 mm with an ocular micrometer.

Ovaries were removed and weighed to the nearest 0.01 g. Two portions of the ovary were sampled: one was stored in buffered 10% formalin in seawater and the other was fixed in Bouin's solution and later transferred to 70% ethanol. The latter portion was then embedded in paraffin, sectioned at 8–10 µm and stained with "one time" trichrome (Gabe, 1958). Ovarian development was described in two ways: 1) the gonadosomatic index was the ratio weight of ovaries : total body weight, multiplied by 100; 2) mean diameter of oocytes was determined from measurements of 30 to 40 of the roundest oocytes from the periphery of the formalin-preserved ovary by using an eyepiece micrometer on a compound microscope.

Ten to 12 males were dissected bimonthly to check for the presence of spermatophores in the vas deferens. The right vas deferens was stored in 10% formalin and later pressed between a slide and coverslip to detect spermatophores under a microscope.

Data were $\log_{10}$-transformed to obtain normality and to reduce heteroscedasticity. Simple correlations and predictive regressions were then calculated for selected life history traits. Outliers were detected by comparing the values of the standardized residuals from the regression line to Student-*t* tabulated values. Slopes and elevations of regressions were compared by analyses of variance (ANOVA) and of covariance (ANCOVA), respectively (Sokal and Rohlf, 1981).

Discriminant analysis was used to determine the existence of two groups in ovarian development data. We then classified each crab into either group on the basis of an objective decision rule (Morrison, 1976). Variation in oocyte diameter and gonadosomatic index were positively correlated with ovary size. Thus, one of the assumptions of discriminant analysis was not met since variance-covariance matrices were not statistically equal even though data were transformed. The discriminant function was used to score individual females. The Games and Howell test (Sokal and Rohlf, 1981) was then used to contrast groups identified by the discriminant analysis.

Results

Gonadal and morphometric maturity

Fifty percent gonadal maturity occurred at 50.2-mm CL in males and at 60.6-mm CL (95% confidence limits: 58.3–62.9) in females (Fig. 2A). Even though the largest females did not carry eggs, we assumed they were mature because their ovaries were normally developed. The smallest male with spermatophores was 49.1 mm CL, whereas the largest one without spermatophores was 70.2 mm CL. The smallest female carrying eggs was 59.7 mm CL and the largest one without eggs and without developed ovaries was 75.1 mm CL.

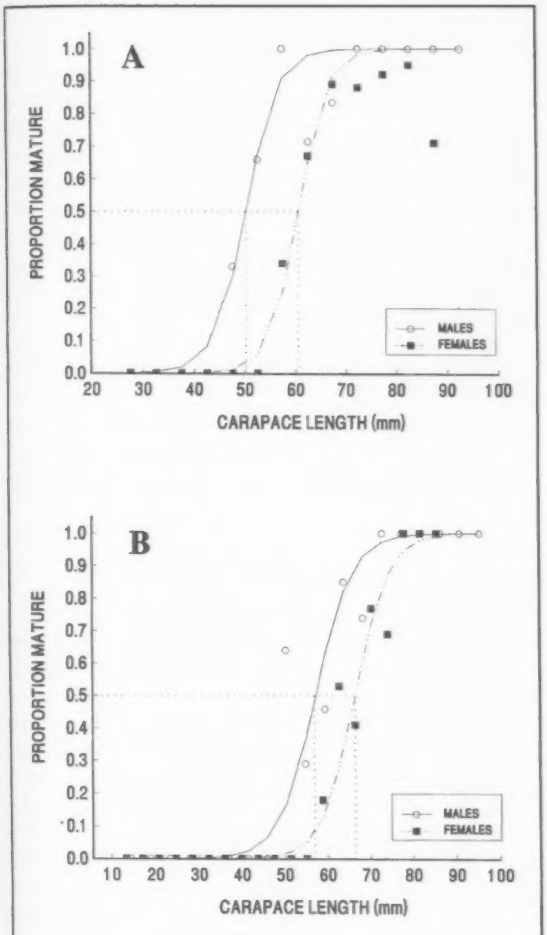

Figure 2

Gonadal and morphometric maturity in *Paralomis granulosa*. Size at 50% maturity is given by the intersection between the calculated logistic function and 0.5 ordinate. (**A**) Proportion of mature *P. granulosa* on the basis of presence of spermatophores in 137 males and presence of eggs or empty egg capsules in 732 females. (**B**) Proportion of mature *P. granulosa* on the basis of allometric growth of chelae.

Size at morphometric maturity was calculated for 851 females (11–88.8 mm CL) and 759 males (6.5–115.1 mm CL). The slope of the regression of chela height on carapace length for juvenile males (<49.1 mm CL; slope=1.012) was significantly less (F=385.7; P<0.001) than that for adult males (>70.2 mm CL; slope=1.473) (Fig. 3). The slope of the regression for known juvenile females (<59.7 mm CL; slope=0.96) was significantly greater (F=223.0; P<0.001) than that of adult females (>75.1 mm CL; slope=0.84). Estimated size at morphometric maturity 50% were 57.0 mm CL (95% confidence limits: 53.9–60.1) for males and 66.5 mm CL (95% confidence limits: 63.4–69.5) for females (Fig. 2B). These results indicated that the method can be applied to both sexes.

Embryogenesis

Embryogenic development was divided into five stages as follows:

Embryonic Stage I (ES I): Spherical or slightly ellipsoidal egg. Yolk bright yellow or orange. A perivitelline space present between the chorionic membrane and the yolk mass. No evidence of division.

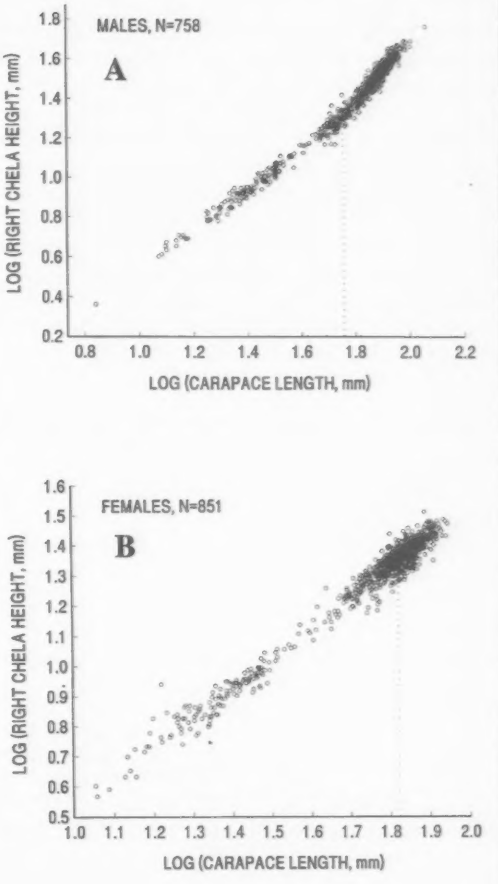

Figure 3

Scattergrams of log right chela height on log carapace length for 758 males (**A**) and 851 females (**B**) of *Paralomis granulosa*. Data for regenerated chelae were removed. Dotted line shows the intersection of juvenile and mature regressions and represents size at maturity: CL = 57 mm for males; CL = 66.5 mm for females.

Embryonic Stage II (ES II): Similar to stage I but a whitish mass of cells visible on yolk surface. This mass is associated with a slight depression in the yolk where the perivitelline space is deeper.

Embryonic Stage III (ES III): A whitish embryo without pigmentation clearly visible, occupying approximately 20% of egg volume. Eye spots clearly outlined. A dorsal organ is present at opposite of embryo (cf. ectodermal thickening of other malacostracan and peracaridan embryos; Anderson, 1982). Abdomen is segmented (5 somites+telson). Telson with 5–7 setae on each of the two lobes. Antennulae and antennae have setae-like projections. Three pairs of appendages are formed posterior to the antennae: mandible, maxillula, and maxilla.

Embryonic Stage IV (ES IV): Egg ellipsoidal; embryo occupies up to 50% of egg volume. Eye spots with ocular pigments. Up to 200 red chromatophores present on carapace. Dorsal organ no longer found.

Embryonic Stage V (ES V): Egg ellipsoidal, pale brown to pale orange. Fully developed zoea clearly visible. Embryo occupying from 50% to almost all of egg volume. Yolk masses reduced and restricted to two dorsal areas in the anterior third of the cephalothorax. All post-mandibular cephalothoracic appendages are differentiated into 3 pairs of maxillipeds and 5 pairs of pereopods, the last one reduced. Telson with 9 setae on each lobe. Heart beat evident.

Duration of embryogenesis and molt stages

Females held in the laboratory were divided into two groups on the basis of embryonic development. One group of females had eggs in ES II from April 1990 up to September 1990 through February 1991, when ES III appeared. ES III did not last longer than two months (in each egg mass) and in May 1991 all eggs were in stage IV. Therefore, these eggs spent autumn and winter in stage II, which suggests an arrest in embryonic development or diapause. The second group of females had clutches with eggs in ES IV in April 1990, which hatched into larvae in October 1990. Post-ovigerous females molted in November 1990, and then extruded and attached eggs in the absence of males. These eggs were unfertile since females have no receptacle to store male gametes (cf. spermathecae in majids). The unfertilized eggs were lost within the next 20 days.

Females collected from the Beagle Channel at any time during the year had egg masses with eggs either in early (I or II) or late (III, IV, or V) stages of development (Table 1; Fig. 4A). ES I and ES II were generally >40% in frequency during the two sampled years. ES III never exceeded 5% in frequency and was observed in February and August 1989, and in April, July, and October 1990. These findings suggest that diapause ends between early winter and late summer, as observed in the laboratory. ES IV and ES V occurred mainly from late autumn to early spring. Post-ovigerous females appeared from late autumn to early spring.

Samples of *P. granulosa* taken from the study area at any given time comprised females in at least two different molt stages: either EIM or AIM occurred with MIM (Table 1, Fig. 4B). PRM females occurred in October and POM females were found in October and November 1989.

Asynchronous embryonic development

A portion of the females (21%; n=100) from the Bécasses Islands, and some from the area near Ushuaia (2%; n=395) carried clutches with eggs at different stages of development: ES II and ES IV or V were found in the same egg mass. This was observed around the Bécasses Islands in August 1990, where 7 females carried eggs in ES II (at least 50% frequency in each clutch) and in ES IV. In October 1990, 12 females had eggs in ES II (at least 50% frequency) and ES V. Two other clutches had eggs in ES II and ES IV. The more advanced eggs would probably hatch earlier than those that were less developed. We could not determine when hatching occurred because commercial fishing around the Bécasses Islands was suspended in October 1990 and the females were not held for further observation.

Table 1

Embryonic development and shell condition of female *Paralomis granulosa* on 20 May 1990 in two localities, 10 km apart. ES=embryonic stages. Postovigerous females have empty egg cases and funiculi attached to the pleopodal setae. MIM=median intermolt; AIM=advanced intermolt. Frequencies for both embryonic development and shell-condition are significantly different between the two localities (G-test for homogeneity on embryonic development = 12.66; P<0.005; G-test for homogeneity on shell condition = 23.45; P<0.005).

	Embryonic development			Shell condition		
Location	ES I, II, and III	ES IV and V	Postovig. females	MIM	AIM	n
Golondrina	60	31	9	64	35	587
Bridges Is.	44	51	5	31	69	84

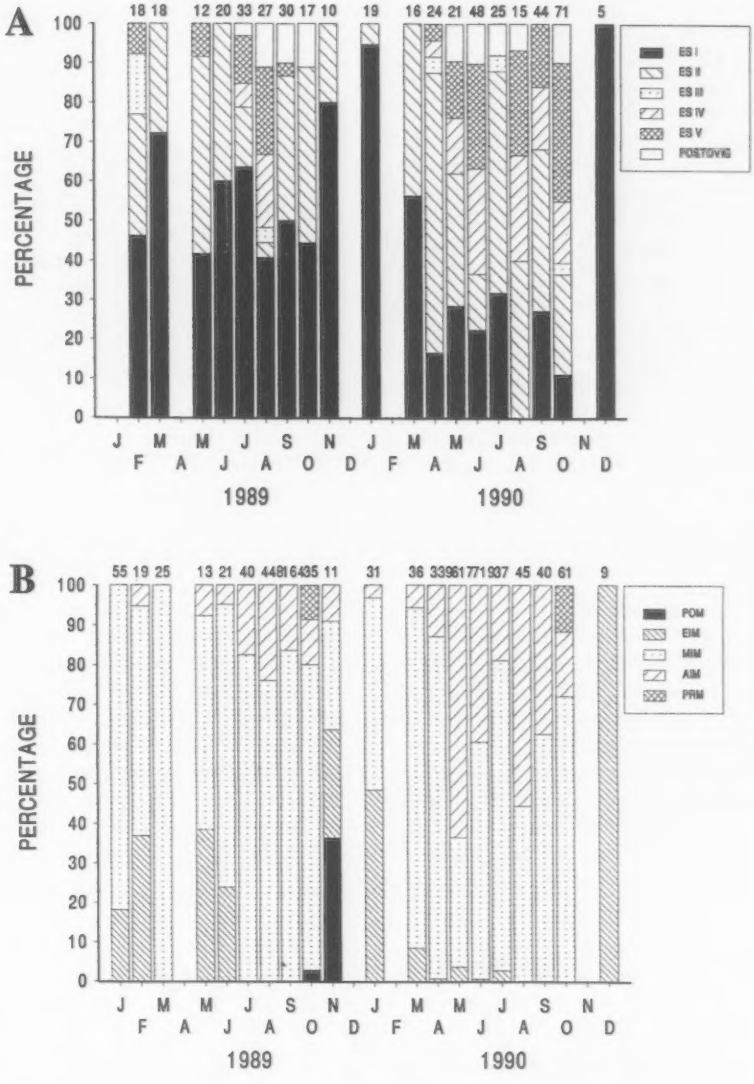
A
PERCENTAGE
18 18 12 20 33 27 30 17 10 19 16 24 21 48 25 15 44 71 5
ES I
ES II
ES III
ES IV
ES V
POSTOVIG
J F M A M J J A S O N D J F M A M J J A S O N D
1989
1990
B
PERCENTAGE
55 19 25 13 21 40 44 81 64 35 11 31 36 33 96 17 71 93 7 45 40 61 9
POM
EIM
MIM
AIM
PRM
J F M A M J J A S O N D J F M A M J J A S O N D
1989
1990

Figure 4

(**A**) Frequency of occurrence of egg stages through two years of sampling. Sample sizes are indicated above bars. Months with no samples are bare. (**B**) Frequency of occurrence of molt stages through two years of sampling. Sample size are indicated above bars. Months with no samples are bare.

Embryonic growth

Eggs from a single clutch increased significantly in size from the ES II (1.71 mm) to ES V (1.96 mm) (paired *t*-test: t=4.604; 3 df; P=0.02) in approximately one year of incubation in the laboratory. Within each asynchronously developing clutch, eggs in ES II (1.87 mm) were significantly smaller than those in ES IV or ES V (1.94 mm) (completely randomized ANOVA; F=22.5; 1,12 df; P<0.001). Egg size at a given stage of development varied significantly among females (F=13.28; 12,12 df; P<0.001). This demonstrates that embryonic growth cannot be generalized from eggs of individual female *P. granulosa*.

Development and maturity of ovaries

Ovarian development of *P. granulosa* was described in detail by Lovrich (1991) and is similar to that of *L. santolla* (Vinuesa, 1984). The only difference with the latter was that oocytes in the ovaries of female *P. granulosa* that had recently molted and spawned were already in an early vitellogenic phase. These oocytes were surrounded by fibrous connective tissue and radiated outwards from the germ strand.

Two groups of females, separated by differences in oocyte diameter (OD) and gonadosomatic index (GSI), were present in virtually all of the sampled months (Figs. 5 and 6). Discriminant analysis on a matrix consisting of CL, CH, OD and GSI (n=220 females sampled during 1990) gave 2 groups. The first included POM, EIM, or MIM females with eggs in ES I and ES II, the second comprised AIM or PRM females with eggs in ES III, ES IV, or ES V. The resulting discriminant function was

$$y = 0.083\ CL + 0.08\ CH - 0.727\ OD - 0.323\ GSI,$$

where coefficients were standardized by the standard deviations within groups. The canonical correlation was 0.798. The reproductive variables OD and GSI dominated the function, whereas morphological measurements contributed negligibly (canonical loadings: CL: 0.17; CH: 0.155; OD: –0.955; GSI: –0.866).

An *a posteriori* classification was generated from the discriminant function. After applying the discriminant function to data for individual females we found that mean scores (i.e., y in function above) were significantly different for each group (Games and Howell test, t'_s=2.49; P<0.01). We thus conclude that there are two distinct groups differing in shell condition and development of ovaries and eggs. These two groups are a key feature of a biennial reproductive cycle.

Females with asynchronous embryonic development were grouped by the discriminant analysis with females in their first year of the reproductive cycle. In fact, for these females, mean GSI was 2.78% in August and 3.38% in October, representing 45 to 55% of maximum GSI (fully developed ovaries in October, Fig. 5), respectively. Their average OD was 1.24 mm in August and 1.36 mm in October, whereas the maximum expected at the end of the reproductive cycle was 1.8 mm (Fig. 6).

Fecundity and reproductive effort

Fecundity was studied in relation to carapace length in the Ushuaia (Fig. 7) and Bécasses Islands. Clutches in late stages (ES IV or ES V) were analyzed separately to avoid underestimations due to possible loss of eggs (Kuris, 1991). Regressions of fecundity on carapace length were significant (Table 2; Fig. 7) and the slopes did not differ between females carrying clutches with eggs in ES I-II, in ES V, or with asynchronously developing eggs (from Bécasses Islands) (F=1.12; P=0.33). ANCOVA indicated that females with ES V eggs or with asynchronously developing eggs had fewer eggs per clutch than those with ES-I and ES-II eggs (F=7.29; P<0.001) (Table 2). Adjusted fecundity was

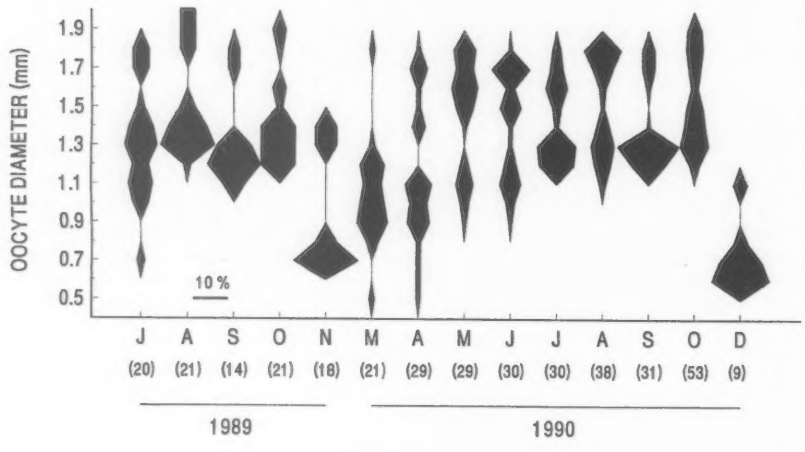

Figure 5
Relative frequency of oocyte diameter for female *Paralomis granulosa* caught in the Beagle Channel. Sample size is indicated in parentheses below each month.

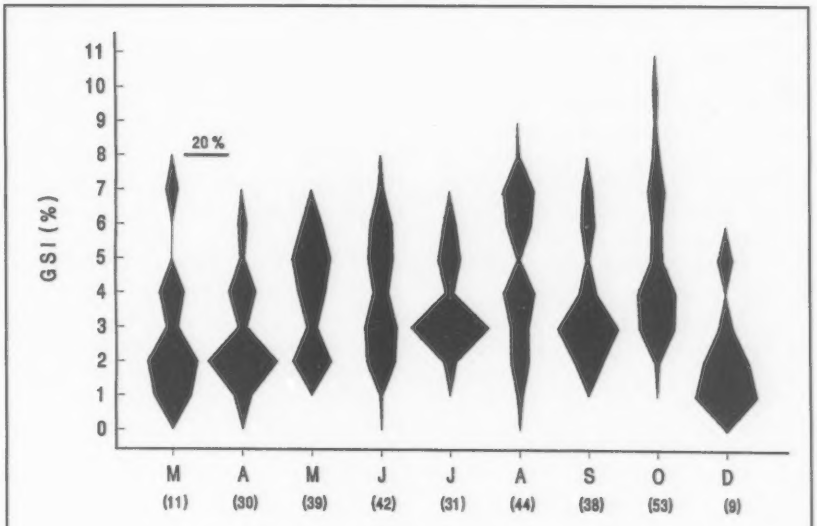

Figure 6

Relative frequency of gonadosomatic index (ratio ovaries weight : body weight * 100) of female *Paralomis granulosa* during 1990. Sample size is indicated in parentheses below each month.

3,688 eggs for females with ES-V eggs and 3,783 eggs for females with asynchronously developing eggs, representing about 88–90% of the estimated 4,201 eggs of females with ES I–II clutches for a constant carapace length (71.0 mm).

The ovaries and clutch of female *P. granulosa* were limited to less than 6–7% of body size. Brood weight varied from 3.67 to 30.39 g and scaled isometrically with weight of body, excluding clutch. Brood weight was at most 7% of female body weight:

$$\log \text{clutch weight} = -0.94 + 0.89 \log \text{weight (body excluding clutch)}$$

(n=196; r^2= 0.303 $F_{regression}$ = 81.99, P<0.001; t-statistic for H_0 slope=1 = –1.122; P=0.26).

In addition, towards the end of the reproductive cycle in October (23rd month), GSI was about 6–7% (Fig. 6).

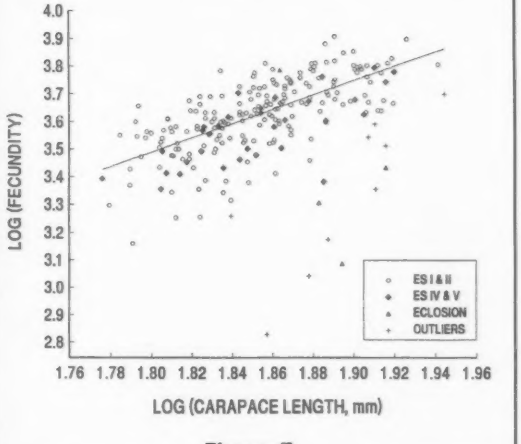

Figure 7

Scattergrams of log fecundity (eggs carried by a female) on log carapace length for *Paralomis granulosa* from Ushuaia area. ES I and II, n=192; ES IV and V, n=32; hatching eggs, n=4; outliers, n=9. The line represents the calculated equation for clutches in ES I and II.

Discussion

Our data indicate that *Paralomis granulosa* has a biennial reproductive cycle. First, female *P. granulosa* molt from late October to November, as evidenced by occurrence of PRM and POM stages (Fig. 4B). We consider that this event marks the beginning of the molt cycle and thus of the reproductive cycle. The frequencies of the different molt stages were consistent with those of the embryonic developmental stages, suggesting that embryogenesis and molting are phased. Embryogenesis lasts 18–22 months and is protracted by a 10-month diapause. Females with ES-I and ES-II eggs were always present throughout the year in the field samples and laboratory. Eggs are extruded from October through November and embryonic development stops or slows at the beginning of cell division (ES II probably representing the diapause stage). After approximately one year of incubation, embryonic development resumes between October and January, and

Table 2

Predictive regression of fecundity (F) on crab size (CL, in mm) in two different locations of the Beagle Channel, during 1989 and 1990. Regressions for different stages of embryonic development (ES) are presented for Ushuaia. R^2=coefficient of determination; F=F-statistic; ***=P<0.001.

Embryo development and location	Equation of regression	n	r^2	F	CL range (mm)
(1) ES I and II (Ushuaia)	$\log F = -1.21 + 2.61 \log CL$	192	0.459	161.3***	60.2–88.0
(2) ES V (Ushuaia)	$\log F = -1.03 + 2.48 \log CL$	32	0.501	33.2***	59.7–83.2
(3) Asynchronous (Bécasses I.)	$\log F = -2.72 + 3.41 \log CL$	43	0.528	45.9***	61.1–78.4

Average log CL = 1.851 (± 0.034)

Adjusted log F (1) = 3.633 (± 0.007)
Adjusted log F (2) = 3.567 (± 0.017)
Adjusted log F (3) = 3.578 (± 0.015)
(1) ≠ (2) & (3), $P < 0.01$; (2) ≠ (3), $P = 0.63$

continues through the next winter, when eclosion occurs. Second, females separated into two groups on the basis of brood development, shell condition, and maturity of ovaries (Table 1; Figs. 5 and 6). In general, females that carried eggs in ES IV and ES V were in advanced molt stages (AIM and PRM) and had fully developed ovaries; by contrast, females with eggs in ES I, ES II, and ES III were in EIM or MIM stages and had small ovaries. Additionally, the presence of oocytes in early vitellogenesis in the recently spawned ovary denotes that oogenesis started before spawning and thus lasts more than two years. In *Lithodes santolla*, oogenesis lasts 24 months (Vinuesa, unpubl. data) while embryogenesis lasts 11 months (Vinuesa, 1984).

The asynchronous embryonic development of *P. granulosa* is a novel feature among lithodid crabs. Our data suggest that some of the eggs within a single clutch develop in 12–14 months and hatch in early summer, while the remainder of the eggs complete their development during the next 10 months. We discount the possibility of a second mating and egg extrusion without molting because 1) there is no seminal receptacle, and chitinous plates cover the gonopores during intermolt, and 2) the ovaries of females with asynchronously developing eggs were in the first year of their cycle. There is clearly a regional effect involved in asynchronous development since females from Ushuaia had more uniformly developed broods. However, with data presently available, we cannot speculate on the causes of this phenomenon.

Since gonadal maturity (presence of gametes) does not necessarily imply morphometric maturity (crabs with differentiated secondary sexual characters), these two terms should be used to define different events in life history that may or may not occur simultaneously. We consider that these concepts are applicable to *P. granulosa* since different sizes at maturity were calculated by using different features (Figs. 2 and 3). Size at maturity for *P. granulosa* tends to increase with increasing latitude (Table 3). This relationship has been noted for *L. santolla* as well (Vinuesa, 1985). By contrast, in the Northern Pacific, size at maturity of lithodid crabs decreases with increasing latitude (Jewett et al., 1985; Somerton and Otto, 1986; Blau, 1990; Otto et al., 1990). Causes of geographical variation in size at maturity are still unknown, but environmental conditions such as bottom temperature may be a factor.

Traps may give biased samples because 1) they select for larger animals (Miller, 1990, but see Blackburn et al., 1990), 2) small crabs and females are excluded

Table 3

Size at maturity (in mm) of *Paralomis granulosa* in different localities. Size at morphometric maturity (SMM) is presented for males and size at gonadal maturity (SGM) for females. Where available, ±95% confidence intervals are presented in parentheses.

Area	Males (SMM)	Females (SGM)
Malvinas I. (Falkland)[1] (51°30'S)	52 (2.15)	46
Seno Otway[2] (53° S)	64	52
Magellan Strait[2] (52°30'S–53°S)	71	62
Beagle Channel (1981–2)[2] (54°55'S)	75	66
Beagle Channel (this study)	57 (3.1)	60.6 (2.3)

[1]Hoggarth, D.D., National Rivers Authority, Welsh Region, Gwyneedd, UK, pers. commun. 1992.
[2]Campodónico et al. 1983.

by larger males, and 3) crabs near ecdysis and berried females are less vulnerable to trapping since they do not feed (Miller, 1990). Size at maturity of *P. granulosa* may be overestimated because the 57.5-mm-CL size class was poorly sampled; this would particularly affect size at gonadal maturity. Also, the frequency of occurrence of females with late embryonic stages, especially those in pre- and postmolt conditions, may be underestimated by trap sampling. Unfortunately, we have no way to assess these possible biases because we have no trawl surveys with which to compare data.

Compared with other shallow-dwelling lithodids, *P. granulosa* has low fecundity and large eggs, resembling in this respect the deep-water species: *Lithodes ferox* (8,000 eggs maximum, 1.97 mm egg diameter; Abelló and Macpherson, 1992); *L. couesi* (5,000, 2.3 mm; Somerton, 1981); *L. murrayi* (4,200, 2.45 mm; Miquel and Arnaud, 1987; Miquel et al., 1985). By contrast, the shallow water *Paralithodes camtschaticus* and *P. platypus* carry up to 350,000 and 280,000 eggs, respectively, with an average egg diameter of 1.2 mm (Matsuura et al., 1971, 1972; Somerton and MacIntosh, 1985). Fecundity of *P. granulosa* was less in females with ES V or with asynchronously developing clutches for a constant carapace length (Table 2), because of egg loss. At the end of embryogenesis about 10–12% of the initial brood was lost. Diseases and egg predators are frequent causes of egg loss in crab species (Kuris, 1991). However we did not find evidence of epibiosis in broods of *P. granulosa*. Exceptionally small broods, outliers in Fig. 7, may have resulted from delayed mating, lack of mates, or to small size of mating males, as occurs in other lithodids (McMullen, 1969; Powell et al., 1973; Paul and Paul, 1990). This question requires further investigation.

As the energy expended on each offspring increases, the number of offspring that parents produce decreases (Smith and Fretwell, 1974). Thus, in two related species similar energetic investment may result in many small or few large offspring. However, considering the two lithodids of the Beagle Channel, one finds that *P. granulosa* biennially produces eggs which are fewer but not larger than those of *L. santolla*, which annually produces up to 59,000 eggs (Guzmán and Campodónico, 1972) of 2.1–2.2 mm in diameter (Vinuesa, 1987).

There is no evidence that the biennial reproductive cycle of *P. granulosa* is more advantageous (i.e., an adaptative strategy) than the annual cycle of *L. santolla*. *Paralomis granulosa* larvae pass through fewer molting events (Campodónico, 1971; Campodónico and Guzmán, 1981); thus mortality due to ecdysis is reduced. Shorter larval development would also reduce predation risks in the plankton. Inhabiting the layer of water closest to bottom (Lovrich, unpubl. data) allows larvae to find refuge and thus reduces losses to predation. We speculate that the lesser fecundity of *P. granulosa* may be partially compensated for by a high survival during their development. In *Paralithodes platypus*, Jensen and Armstrong (1989) interpreted biennial reproduction as a consequence of physiological and energetic constraints incurred by the species in a harsh environment.

King crab species can be categorized into three groups on the basis of their reproductive cycles: *Paralithodes camtschaticus* and *Lithodes santolla* spawn annually, *Paralomis granulosa* and *Paralithodes platypus* (except primiparous females) spawn biennially, and finally *L. aequispina, L. couesi*, and *L. ferox* spawn asynchronously. All *Lithodes* species, with the exception of *L. santolla*, inhabit deep waters whereas *Paralithodes* species inhabit shallow waters. Otto and Cummiskey (1985) hypothesized that king crabs inhabiting shallow waters (*L. santolla, P. camtschaticus*, and *P. platypus*) spawn synchronously during spring while deep-sea king crabs (*L. aequispina* and *L. couesi*) have protracted spawning periods. These authors suggest that this pattern could be related to a dependence on food sources by shallow water species. *Paralomis granulosa* inhabits shallow waters and spawns synchronously every two years, but we suppose that in this species synchronicity is not related to food dependence because larval hatching occurs mainly during winter when neither food nor potential competitors are abundant (Lovrich, unpubl. data).

Paralomis is a deep-water genus (Takeda et al. 1984; Macpherson, 1988) and *P. granulosa* is the only species that inhabits shallow waters. This species probably colonized the Beagle Channel relatively recently, i.e., 8,500 years ago after the last deglaciation occurred (Rabassa et al., 1986). This species still retains certain features of its deep-water relatives: low fecundity, large eggs, protracted reproductive cycle, and independence of larval hatching from food availability.

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The assumption of constant selectivity and the stock assessment for widow rockfish, *Sebastes entomelas*

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Abstract.—Modern methods for fish stock assessment are often based on age-structured models that separate each coefficient of fishing mortality at age into a time-specific factor (the rate of fishing mortality on the fully exploited age-classes) and an age-specific factor (a selectivity coefficient that measures the relative vulnerability of the particular age-class). The assumption that the selectivity coefficients are constant through time greatly simplifies the assessment process because it allows for a reduction in the number of unknown parameters. However, if the assumption is incorrect, it can lead to incorrect estimates of stock status.

The most recent stock assessment for Pacific widow rockfish (*Sebastes entomelas*) was based on the untested assumption that the selectivity coefficients have not changed over the years. This assessment was derived from an analysis of catch-at-age data by using an assessment method known as the Stock Synthesis program. The work described here examined the sensitivity of the assessment results to the assumption of constant selectivity. Simulation experiments with the Stock Synthesis program showed that the stock size estimates for widow rockfish can be highly sensitive to modest changes in selectivity. Experiments with two other assessment techniques, which also assume constant selectivity (the CAGEAN program of Deriso, Quinn, and Neal and the multiplicative catch-at-age model of Shepherd and Nicholson), showed that these methods are similarly sensitive to changes in selectivity.

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In general, individual fish in a stock are not equally likely to be caught and different age-classes of fish do not experience identical rates of fishing mortality. In the fisheries literature this phenomenon is usually described as "selectivity" or "availability" or "partial recruitment" (Megrey, 1989). In some instances selectivity results from the physical properties of the fishing gear. For example, younger and smaller fish may pass unharmed through the meshes of a trawl, whereas older and larger individuals may sense and avoid an approaching net. Alternatively, selectivity can result when different age-classes of fish occupy geographic regions that are not fished with the same intensity. If younger fish are offshore and older ones are inshore, for example, then the age distribution of fish in the catch will depend not just on the stock's age distribution but also on the fishing locations. Selectivity coefficients, which measure the relative influence of fishing on the age structure of the stock, are fundamental parameters in the analysis of catch-at-age data.

Many stock assessment procedures attempt to reconcile observations of catch-at-age with an underlying age-structured population model and thereby reconstruct the demographic history of the stock (Megrey, 1989). If different age-classes experience the same relative susceptibility to fishing each year, then one can model each annual age-specific rate of fishing mortality as the simple product ($S_a \cdot F_y$) of an age effect (the selectivity coefficient, S_a) and a year effect (the fishing mortality coefficient, F_y). Because there are an infinite number of (S_a, F_y) pairs that correspond to a given age-specific rate of fishing mortality, the selectivity coefficient for at least one age class must be assumed constant. If the largest S_a is set equal to one, then the F_y values correspond to the rate of fishing mortality on the fully exploited age classes.

Formulating the fishing mortality coefficient as the product of a year-effect and an age-effect greatly simplifies an analysis of catch-at-age data because it reduces the number of essential parameters. For example, if the catch-at-age matrix contains data for A ages and Y years, and if the selectivity coefficients are constant for all years, then there are only $(A+Y)$ unknown parameters. However, if the selectivity coefficients change every year, then there are $(A \cdot Y)$ unknown parameters.

Constant selectivity, and the consequent separability of fishing mortality into age and year effects, is a fundamental assumption for numerous stock assessment procedures, including separable Virtual Population Analysis (Pope and Shepherd, 1982), the CAGEAN program (Deriso et al., 1985, 1989), the multiplicative model of Shepherd and Nicholson (1986, 1991), and the Stock Synthesis program (Methot 1989, 1990). Fish stocks that have recently been as-

sessed by using one or more of these procedures include Pacific halibut (*Hippoglossus stenolepis*) in the northeast Pacific (IPHC, 1991), walleye pollock (*Theragra chalcogramma*) in the Gulf of Alaska (Megrey, 1991), Dover sole (*Microstomus pacificus*) along the U.S. west coast (Turnock and Methot, 1991), and scad (*Trachurus trachurus*) from Atlantic waters off Spain and Portugal (Borges, 1990).

Despite the widespread application of assessment methods that are based on the notion that selectivity is time-invariant, I know of no published studies that examine the sensitivity of these assessment procedures to violations of the constant selectivity assumption. It appears that often these assessment methods are applied without first verifying that selectivity was constant for the stock being assessed. Gudmundsson (1986) recommended extensive analysis to avoid mis-specifying the catch-at-age model (for example, incorrectly assuming that selectivity was constant), and he developed a least-squares technique for testing the separability assumption. However, his methodology does not seem to be used widely.

Several published papers document variations in selectivity through time. Houghton and Flatman (1981) examined selectivity coefficients for cod (*Gadus morhua*) in the west-central North Sea and found significant changes in the "exploitation pattern," which they attributed to shifts in the fishing pressure exerted by different segments of the fleet. Gudmundsson (1986) speculated that changes in fish size-at-age coupled with variations in the composition of the fishing gear caused changes in selectivity for the Icelandic stock of cod (*Gadus morhua*). Gordoa and Hightower (1991) analyzed data from the fishery for Cape hake (*Merluccius capensis*) off southwestern Africa and ascribed significant shifts in selectivity to the fishermen's targeting on strong year classes.

The work described here has a different focus. In this paper I do not examine how selectivity in a fishery has varied. Instead, I investigate whether the stock assessment program used to evaluate a particular stock's status is robust to changes in selectivity. For this exercise I analyzed the stock assessment for widow rockfish (*Sebastes entomelas*), an economically important component of the complex of Sebastes species found along the Pacific coast of North America. Gunderson (1984) described the history and characteristics of the U.S. fishery for widow rockfish off the coasts of Washington, Oregon, and California.

In this paper, I demonstrate that the stock size estimates for widow rockfish, which are based on the untested assumption that selectivity has been constant from year to year, can be seriously biased if the assumption is violated. Furthermore, I test two other assessment methods that also use the constant selectivity assumption and show that they produce similarly biased results. Finally, I establish that comparable problems with bias can arise in the assessment results for other fish stocks, whose biological characteristics differ significantly from widow rockfish.

Methods

One technique for testing the reliability of an estimation procedure is to produce artificial data sets with known characteristics and then to estimate the parameter values from which the data were derived. I used this approach to determine whether certain stock assessment methods were sensitive to violations of the constant selectivity assumption. First, I simulated catch-at-age data for a fish stock in which the selectivity coefficients were changing slowly from year to year. Next, I used the assessment programs to analyze the catch-at-age data and to estimate stock biomass and abundance-at-age. Finally, I measured the bias of the estimates by calculating the relative errors of the estimates. The relative error of an estimate is the difference between the estimate and its true value; all divided by the true value.

Sensitivity of the Stock Synthesis program when applied to data for widow rockfish

To investigate the sensitivity of the stock assessment results for widow rockfish to the assumption of constant selectivity, I developed a spreadsheet model to generate artificial catch-at-age data, which I then analyzed using the Stock Synthesis program. The Stock Synthesis program has been used by the Pacific Fisheries Management Council (PFMC) since 1990 to appraise the status of many of the Pacific groundfish stocks (PFMC, 1990). Methot (1989, 1990) documented the principles and equations underlying the Stock Synthesis program.

The spreadsheet model simulates the characteristics of an age-structured population and employs the same equations as the Stock Synthesis program for describing the temporal progressions in abundance-at-age, biomass, catch-at-age, and total catch. The model uses parameters for mortality and growth that are similar to those observed in the U.S. stock of widow rockfish (Table 1). I generated values for abundance-at-age and catch-at-age for 10 years and 20 age classes, ages 4 through 22, as well as age 23 and older (Table 2). In 1989, almost 95% of the U.S. coast-wide landings of widow rockfish were fish between the ages of 5–10 years; about 3% of the landings were fish older than 15 years (Hightower and Lenarz, 1990).

Table 1
Parameters for simulating a stock of widow rockfish: annual recruitment was 10^6 fish per year; natural mortality[1] was 0.15 per year. Selectivity parameters[2] (Curve B (Fig. 1), 100% selection at age 8): lower inflection age was 6.0 years; lower slope was 2.5 per year; upper inflection age was 12.0 years; and the upper slope was 0.3 per year.

Age (years)	Selectivity coefficients (%)	Weight[3] (kg)
4	0.8	0.549
5	8.9	0.662
6	56.2	0.780
7	99.0	0.936
8	100.0	1.025
9	93.1	1.150
10	84.6	1.255
11	75.2	1.400
12	65.5	1.427
13	55.7	1.596
14	46.4	1.798
15	37.9	1.899
16	30.3	1.965
17	23.9	1.965
18	18.6	2.008
19	14.3	2.099
20	10.9	2.031
21	8.2	2.704
22	6.2	2.299
23+	4.7	2.388

[1]From Hightower and Lenarz (1990).
[2]The equation for the double-logistic selection curve and the meaning of these parameters are described in Methot (1990).
[3]From Barss and Echeverria (1987).

In the first set of experiments, I examined combinations of three factors to determine how they affect bias in the estimates from the Stock Synthesis program. They were 1) the age of full selection was either gradually increasing or decreasing from year to year, or it varied randomly; 2) the annual fishing mortality coefficients were either increasing, decreasing, or constant; and 3) the program was either given the true values of annual recruitment or it was required to estimate these values. For simplicity, I limited my experiments to these three factors, although undoubtedly there are others that can also have significant effects. Examples are trends in annual recruitment, the level of natural mortality, or the shape of the selectivity curve.

True values for the selectivity coefficients were generated from a double logistic function (Methot, 1990) and were similar to those reported for widow rockfish in Hightower and Lenarz (1990). The strongly domed shape of the selectivity curve (Fig. 1), which may be due to the movement of older individuals into deeper, less heavily fished waters, seems to be a common feature for many of the groundfish stocks in the U.S. Pacific Northwest.

To simulate temporal changes in selectivity, I shifted the selectivity coefficients forward or backward by one age class (Fig. 1). When selectivity increased, 100% selection occurred at age 7 for the first three years (Curve A), at age 8 for the next four years (Curve B), and at age 9 for the last three years (Curve C). When selectivity decreased, full selection occurred at age 9 for the first three years, at age 8 for the next four years, and at age 7 for the last three years. To measure the effects of "random" changes in selectivity, I generated data sets for 10 trials. Selectivity in the first year of each trial always followed selection curve B, but the sequence of curves that applied in the subsequent years came from a random shuffling of the sequence AAABBBCCC (Fig. 2). Curve A applied in three randomly chosen years, curve B applied in three other randomly selected years, and curve C applied in the remaining three years. I did not examine other forms of change in selectivity, such as variation in the basic shape of the curve.

When simulating an increasing trend in fishing mortality, the fishing mortality coefficients changed linearly from 0.10 to 0.28 per year, at increments of 0.02 per year. When the trend was decreasing, the fishing mortality coefficients varied from 0.28 to 0.10 per year, at increments of –0.02 per year. When there was no trend in fishing mortality, the fishing mortality was 0.20 per year, which is approximately the rate of fishing that reduces the reproductive output from this simulated stock to 35% of its unexploited level when full selection is at age 8 years (Fig. 1, Curve B).

All methods for analyzing catch-at-age data require additional information with which to tune the analysis and thereby resolve a basic indeterminacy in the model for catch[1] (Shepherd and Nicholson, 1986). In the most recent assessment for widow rockfish, Hightower and Lenarz (1990) tuned the Stock Synthesis analysis to a single fishing mortality coefficient, but in many contemporary assessments of other Pacific groundfish stocks the Stock Synthesis runs have been tuned to estimates of abundance-at-age or biomass from research vessel surveys. In the sensitivity analysis, I gave the Stock Synthesis program auxiliary data for tuning either in the form of the true annual fishing mortality coefficients or the true proportions-at-age.

Sensitivity of other stock assessment programs that assume constant selectivity

To confirm that the assumption of constant selectivity, rather than some unique feature of the Stock Synthesis program, was responsible for any bias in the results, I experimented with two other

[1]Catch-at-age is approximately equal to the product of stock abundance-at-age and fishing mortality-at-age. If only catch data are available, one cannot distinguish between a case of large abundance and low fishing mortality versus one of small abundance and high fishing mortality.

Table 2

Simulated widow rockfish abundance and catch data. (**A**) Selectivity increasing, fishing mortality constant (0.20 year). (**B**) Selectivity decreasing, fishing mortality constant (0.20/year). Selection curves are indicted in parentheses.

A	Year									
Age	1981 (A)	1982 (A)	1983 (A)	1984 (B)	1985 (B)	1986 (B)	1987 (B)	1988 (C)	1989 (C)	1990 (C)
Initial population size (1000's of fish)										
4	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
5	845.6	845.6	845.6	845.6	859.3	859.3	859.3	859.3	860.6	860.6
6	650.4	650.4	650.4	650.4	715.0	726.6	726.6	726.6	738.4	739.5
7	459.3	459.3	459.3	459.3	500.3	550.0	558.9	558.9	614.5	624.4
8	323.7	323.7	323.7	323.7	324.3	353.3	388.4	394.7	429.9	472.6
9	231.3	231.3	231.3	231.3	228.1	228.5	249.0	273.7	278.7	303.6
10	168.1	168.1	168.1	168.1	165.2	163.0	163.3	177.9	192.9	196.4
11	124.4	124.4	124.4	124.4	122.1	120.1	118.4	118.7	127.1	137.8
12	94.0	94.0	94.0	94.0	92.1	90.4	88.9	87.7	86.3	92.4
13	72.3	72.3	72.3	72.3	70.9	69.6	68.3	67.1	64.9	63.9
14	56.7	56.7	56.7	56.7	55.7	54.6	53.6	52.6	50.7	49.0
15	45.3	45.3	45.3	45.3	44.5	43.7	42.8	42.0	40.5	39.0
16	36.7	36.7	36.7	36.7	36.1	35.5	34.9	34.2	33.0	31.8
17	30.1	30.1	30.1	30.1	29.7	29.3	28.8	28.2	27.3	26.3
18	25.0	25.0	25.0	25.0	24.7	24.4	24.0	23.6	22.9	22.1
19	20.9	20.9	20.9	20.9	20.7	20.5	20.2	19.9	19.4	18.8
20	17.6	17.6	17.6	17.6	17.5	17.3	17.1	16.9	16.5	16.1
21	14.9	14.9	14.9	14.9	14.8	14.7	14.6	14.4	14.1	13.8
22	12.7	12.7	12.7	12.7	12.6	12.5	12.5	12.3	12.1	11.9
23+	74.3	74.3	74.3	74.3	74.1	73.9	73.7	73.4	72.8	72.2
Catch (1000's of fish)										
4	16.30	16.30	16.30	1.49	1.49	1.49	1.49	0.13	0.13	0.13
5	83.61	83.61	83.61	13.78	14.01	14.01	14.01	1.28	1.28	1.28
6	108.74	108.74	108.74	64.31	70.70	71.85	71.85	11.85	12.04	12.06
7	77.51	77.51	77.51	76.78	83.64	91.95	93.44	55.26	60.75	61.74
8	51.17	51.17	51.17	54.62	54.73	59.62	65.54	65.98	71.88	79.02
9	33.49	33.49	33.49	36.56	36.06	36.13	39.36	46.18	47.03	51.23
10	21.85	21.85	21.85	24.34	23.93	23.60	23.65	28.12	30.49	31.05
11	14.21	14.21	14.21	16.18	15.88	15.61	15.40	17.19	18.41	19.96
12	9.22	9.22	9.22	10.73	10.52	10.33	10.15	11.40	11.21	12.01
13	5.96	5.96	5.96	7.10	6.96	6.83	6.70	7.67	7.42	7.29
14	3.85	3.85	3.85	4.68	4.59	4.50	4.41	5.16	4.97	4.81
15	2.48	2.48	2.48	3.07	3.02	2.96	2.90	3.46	3.34	3.22
16	1.59	1.59	1.59	2.01	1.98	1.94	1.91	2.32	2.23	2.15
17	1.02	1.02	1.02	1.31	1.29	1.27	1.25	1.54	1.49	1.44
18	0.65	0.65	0.65	0.85	0.84	0.83	0.81	1.02	0.99	0.96
19	0.42	0.42	0.42	0.55	0.54	0.54	0.53	0.67	0.66	0.64
20	0.27	0.27	0.27	0.35	0.35	0.35	0.34	0.44	0.43	0.42
21	0.17	0.17	0.17	0.23	0.23	0.22	0.22	0.29	0.28	0.28
22	0.11	0.11	0.11	0.15	0.14	0.14	0.14	0.19	0.18	0.18
23+	0.48	0.48	0.48	0.64	0.64	0.64	0.63	0.84	0.84	0.83

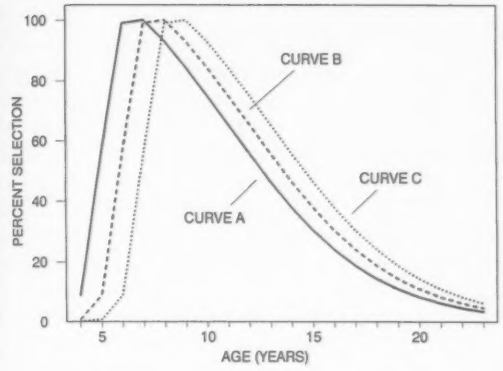
Table 2 (Continued)

B	Year									
Age	1981 (C)	1982 (C)	1983 (C)	1984 (B)	1985 (B)	1986 (B)	1987 (B)	1988 (A)	1989 (A)	1990 (A)
Initial population size (1000's of fish)										
4	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
5	860.6	860.6	860.6	860.6	859.3	859.3	859.3	859.3	845.6	845.6
6	739.5	739.5	739.5	739.5	727.7	726.6	726.6	726.6	661.0	650.4
7	625.3	625.3	625.3	625.3	568.8	559.8	558.9	558.9	513.1	466.7
8	481.0	481.0	481.0	481.0	441.6	401.7	395.3	394.7	393.9	361.6
9	339.7	339.7	339.7	339.7	339.0	311.2	283.1	278.5	282.0	281.4
10	239.4	239.4	239.4	239.4	242.7	242.2	222.3	202.2	202.4	205.0
11	171.0	171.0	171.0	171.0	174.0	176.4	176.0	161.6	149.8	149.9
12	124.3	124.3	124.3	124.3	126.6	128.8	130.6	130.3	122.0	113.1
13	92.0	92.0	92.0	92.0	93.8	95.6	97.3	98.6	100.3	93.9
14	69.5	69.5	69.5	69.5	70.9	72.3	73.6	74.9	77.3	78.7
15	53.5	53.5	53.5	53.5	54.5	55.6	56.7	57.7	59.7	61.7
16	42.0	42.0	42.0	42.0	42.7	43.5	44.3	45.2	46.8	48.4
17	33.5	33.5	33.5	33.5	34.0	34.6	35.2	35.9	37.1	38.4
18	27.1	27.1	27.1	27.1	27.5	27.9	28.4	28.9	29.8	30.8
19	22.3	22.3	22.3	22.3	22.5	22.8	23.1	23.5	24.2	24.9
20	18.5	18.5	18.5	18.5	18.6	18.8	19.1	19.3	19.8	20.4
21	15.4	15.4	15.4	15.4	15.5	15.7	15.8	16.1	16.4	16.8
22	13.0	13.0	13.0	13.0	13.1	13.2	13.3	13.4	13.6	13.9
23+	73.4	73.4	73.4	73.4	73.7	73.9	74.2	74.6	75.2	75.9
Catch (1000's of fish)										
4	0.13	0.13	0.13	1.49	1.49	1.49	1.49	16.30	16.30	16.30
5	1.28	1.28	1.28	14.03	14.01	14.01	14.01	84.97	83.61	83.61
6	12.06	12.06	12.06	73.12	71.95	71.85	71.85	121.48	110.50	108.74
7	61.83	61.83	61.83	104.55	95.10	93.58	93.44	94.32	86.59	78.76
8	80.42	80.42	80.42	81.17	74.52	67.78	66.70	62.40	62.27	57.17
9	57.32	57.32	57.32	53.70	53.59	49.20	44.75	40.34	40.84	40.75
10	37.84	37.84	37.84	34.66	35.14	35.07	32.20	26.29	26.31	26.64
11	24.77	24.77	24.77	22.23	22.61	22.93	22.88	18.45	17.10	17.12
12	16.16	16.16	16.16	14.19	14.46	14.71	14.91	12.79	11.97	11.09
13	10.51	10.51	10.51	9.03	9.21	9.38	9.54	8.13	8.27	7.74
14	6.82	6.82	6.82	5.73	5.84	5.96	6.07	5.08	5.24	5.34
15	4.41	4.41	4.41	3.63	3.69	3.77	3.84	3.16	3.27	3.37
16	2.84	2.84	2.84	2.29	2.33	2.38	2.43	1.96	2.03	2.10
17	1.83	1.83	1.83	1.45	1.47	1.50	1.53	1.22	1.26	1.30
18	1.18	1.18	1.18	0.92	0.93	0.95	0.96	0.76	0.78	0.81
19	0.75	0.75	0.75	0.58	0.59	0.60	0.61	0.47	0.48	0.50
20	0.48	0.48	0.48	0.37	0.37	0.38	0.38	0.29	0.30	0.31
21	0.31	0.31	0.31	0.23	0.24	0.24	0.24	0.18	0.19	0.19
22	0.20	0.20	0.20	0.15	0.15	0.15	0.15	0.12	0.12	0.12
23+	0.84	0.84	0.84	0.63	0.63	0.64	0.64	0.48	0.48	0.49

assessment methods that also use the assumption of constant selectivity. I analyzed the simulated catch-at-age data with the CAGEAN program (CAGEAN-PC, version 4, release 2) of Deriso et al. (1985, 1989) and with the multiplicative catch-at-age model of Shepherd and Nicholson (1986, 1991).

The Stock Synthesis program and the CAGEAN program use similar approaches for modeling catch-at-age, but they differ in their assumptions about variability in the observed data. Stock Synthesis assumes a multinomial error structure for the catch-at-age data. If $\hat{p}_a$ is the predicted proportion of age class (a) captured, then the variance associated with a random observation of p_a is proportional to $\hat{p}_a \cdot (1-\hat{p}_a)$. CAGEAN, however, assumes a lognormal error structure. If $\hat{c}_a$ is the predicted number of fish caught of age class (a), then $\log_e(c_a/\hat{c}_a)$ is normally distributed with a zero mean and a constant variance.

The multiplicative catch-at-age model is essentially an approximation to the catch model that underlies



SELECTION TREND	CURVE	YEAR 1 2 3 4 5 6 7 8 9 10
INCREASING	A	
	B	
	C	
DECREASING	A	
	B	
	C	

Figure 1

Selectivity curves used in the simulations. To simulate the effects of changing selectivity, the selectivity curve was either shifted towards older fish through time (increasing selectivity) or towards younger fish (decreasing selectivity).

both the Stock Synthesis and CAGEAN programs. Unlike the other two assessment procedures, the multiplicative model estimates relative, rather than absolute, abundance. To conduct the multiplicative catch-at-age analyses, I used the GLIM statistical program (Baker and Nelder, 1985) and assumed a log-normal error structure.

In the experiments with CAGEAN and the multiplicative catch-at-age model, I used a subset of the data from the earlier experiments with the Stock Synthesis program. Two sets of catch-at-age data were analyzed, one from a population with selectivity shifting to older ages (selectivity increasing) and fishing mortality constant (Table 2A), the other from a population with selectivity shifting to younger ages (selectivity decreasing) and fishing mortality constant (Table 2B). I tuned the CAGEAN program to the true fishing mortality coefficients, and constrained the multiplicative catch-at-age analysis to have a trend of zero in the annual fishing mortality coefficients. The experiments here correspond to the cases examined earlier in which the Stock Synthesis program was tuned to fishing mortality and recruitment was estimated.

Sensitivity of the Stock Synthesis program when applied to data from a heavily exploited stock

To determine whether the results from the experiments with a simulated stock of widow rockfish would apply to fish stocks with different biological characteristics, I generated two additional data sets, one from a population with selectivity shifting to older ages (selectivity increasing), the other from a population with selectivity shifting to younger ages (selectivity decreasing). Both simulated populations, which suffered an instantaneous natural mortality rate of 0.30 per year and an instantaneous fishing mortality rate of 0.60 per year, had significantly fewer old animals compared to the populations in the previous simulations. I analyzed the two data sets with the Stock Synthesis program, with tuning to the true fishing mortality coefficients, and with recruitment estimated.

Results

The assessment programs that I investigated all produce a wide variety of estimates, including selectivity coefficients and matrices of abundance and catch by age and year. Rather than evaluating bias for all estimates, my analysis focussed on estimates of annual stock biomass, numerical abundance, and recruitment. Of special importance to a stock assessment scientist or fishery manager is the bias in the estimate of average biomass for the final year of a data series. This estimate is approximately the biomass estimate on which the catch quota for the next year is based[2]. If the estimate of average biomass in the final year is, say, 20% too high, then the quota will be roughly 20% too high; if the estimate is 10% too low, the quota will also be about 10% too low.

Selectivity of the Stock Synthesis program when applied to data for widow rockfish

The results of the experiments with the Stock Synthesis program and the data for the simulated stock of widow rockfish suggest that some of the assessment results can be highly sensitive to slight trends in selectivity. For example, when selectivity shifted towards younger ages, the biomass estimate for the final year of the series was 74% too high (Table 3A; selectivity decreasing, tuned to fishing mortality, fishing mortal-

[2]The annual catch quota is derived from an estimate of the biomass at the end of the previous year, plus an appropriate amount for the new recruitment. In practice this differs little from the estimate of average biomass in the final year.

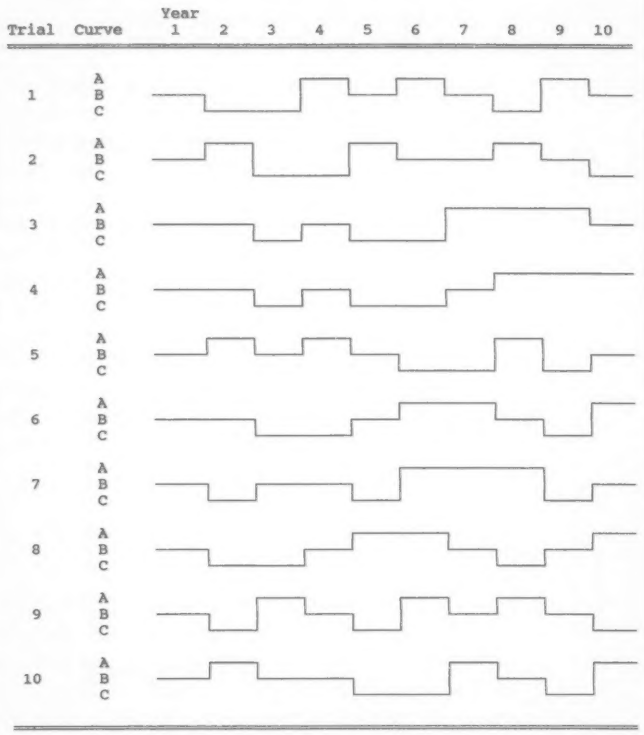


Figure 2

Simulations with random variation in selectivity. The effects of random variation in selectivity were simulated by shuffling the sequence of selectivity curves (AAABBBCCC, Fig. 1) that applied in each of the nine years after the first. The ten sequences shown here were used.

ity constant, and recruitment estimated), and the estimated age distribution in the final year was grossly incorrect (Fig. 3A). The estimated numbers of five-year-old to seven-year-old fish were much too high and the numbers of fish 15 years and older were all slightly too high. When selectivity shifted towards older ages, the biomass estimate for the final year was as much as 59% too low (Table 3A; selectivity increasing, tuned to fishing mortality, fishing mortality increasing, and recruitment estimated) and the program underestimated the numbers of very young and very old fish (Fig. 3B).

In these experiments, bias in a particular estimate was not a simple linear function of the factors examined, but instead involved complicated interactions between factors. Nevertheless, some general effects seemed to apply. When the Stock Synthesis program was tuned to fishing mortality and used to estimate recruitment, shifts in selectivity towards older ages (selectivity increasing) always induced negative bias in the estimates of average biomass, and shifts in selectivity towards younger ages (selectivity decreasing) always induced positive bias (Table 3, A and B). When the recruitment values were known or tuning to the proportion-at-age data was used, however, trends in selectivity had no consistent effect on the direction of bias. Estimation of recruitment values, often, but not always, increased the magnitude of the bias in the estimates of biomass (Table 3, A and B) and abundance (Table 3C). Tuning to proportion-at-age data, instead of to annual fishing mortality coefficients, often decreased the amount of bias in the estimates of biomass, abundance, and recruitment. Bias in these estimates usually was smallest when the trend in fishing mortality was increasing and largest when the trend in fishing mortality was decreasing.

When the Stock Synthesis program estimated recruitment, improvements in the fit were observed relative to those obtained when recruitment values were known (Table 3D). To the assessment scientist interpreting these results, the improved fit would suggest that the program had provided better estimates, when, in fact, the estimates were more biased and less reliable. When selectivity shifted toward older fish, there was a systematic change from year to year in the catch-at-age data, which the program attempted to match by imposing a decreasing trend in recruitment (Fig. 4). When selectivity shifted towards younger fish, the program imposed an increasing trend in recruitment. Similar distortions occurred when the program was tuned to the true proportion-at-age data. This last result suggests that using age-frequency data from research vessel surveys will not eliminate the bias induced by changes in selectivity, even though survey data may not be subject to the changes in selectivity that the fishery might experience.

When selectivity varied randomly (Table 4), the magnitude of the bias in the estimate of the final year's average biomass was usually less than what occurred when selectivity had a trend (Table 3A), but the general patterns seen in the earlier experiments remained.

Table 3

Sensitivity analysis of the Stock Synthesis program. (**A**) Bias[1] in the estimated average biomass in the final year. (**B**) Average and standard deviation[2] of the bias in the estimates of annual average biomass. (**C**) Average and standard deviation of the bias in the estimates of annual numerical abundance. (**D**) Average and standard deviation of the bias in the estimates of annual recruitment[3] and the improvement in fit[4] when recruitment was estimated.

A

	Increasing selectivity		Decreasing selectivity	
	Known recruitment %	Estimated recruitment %	Known recruitment %	Estimated recruitment %
Tuned to fishing mortality				
F increasing	−1.0	−58.8	−1.2	49.8
F decreasing	16.9	−54.4	13.6	72.0
F constant	12.1	−54.0	10.4	74.3
Tuned to proportion-at-age				
F increasing	3.4	−26.1	−2.9	1.0
F decreasing	23.3	−24.6	11.7	5.7
F constant	17.3	−14.0	7.4	5.2

B

	Increasing selectivity				Decreasing selectivity			
	Known recruitment		Estimated recruitment		Known recruitment		Estimated recruitment	
	Mean (%)	SD (%)	Mean (%)	SD (%)	Mean (%)	SD (%)	Mean (%)	SD (%)
Tuned to fishing mortality								
F increasing	3.9	2.1	−32.5	12.7	−4.5	1.3	24.6	16.2
F decreasing	46.0	21.6	−20.8	18.2	21.4	8.0	63.3	18.8
F constant	29.5	11.5	−20.5	17.0	11.8	4.0	53.2	18.2
Tuned to proportion-at-age								
F increasing	4.5	0.5	−12.1	8.4	−3.8	0.4	−6.3	6.9
F decreasing	51.7	20.2	7.6	20.5	24.9	9.6	9.1	11.0
F constant	32.0	10.0	10.8	15.2	13.7	4.9	4.3	7.9

C

	Increasing selectivity				Decreasing selectivity			
	Known recruitment		Estimated recruitment		Known recruitment		Estimated recruitment	
	Mean (%)	SD (%)	Mean (%)	SD (%)	Mean (%)	SD (%)	Mean (%)	SD (%)
Tuned to fishing mortality								
F increasing	2.9	3.0	−27.3	20.4	−2.1	3.4	23.6	27.7
F decreasing	23.1	14.0	−24.1	24.2	8.4	3.5	40.9	27.7
F constant	15.8	8.9	−21.5	24.3	4.7	2.3	38.5	29.9
Tuned to proportion-at-age								
F increasing	3.5	1.6	−10.8	14.8	−2.5	1.6	−3.8	14.0
F decreasing	26.8	13.3	−4.4	22.8	10.2	4.5	−1.3	13.6
F constant	17.7	7.9	1.6	19.7	5.6	2.1	−1.2	13.2

Table 3 (Continued)

D	Increasing selectivity			Decreasing selectivity		
	Mean (%)	SD (%)	Improvement in fit (%)	Mean (%)	SD (%)	Improvement in fit (%)
Tuned to fishing mortality						
F increasing	–30.0	45.3	45.7	24.3	89.8	29.5
F decreasing	–38.0	42.1	56.7	29.8	95.7	32.2
F constant	–32.8	45.2	51.9	30.7	96.0	31.6
Tuned to proportion-at-age						
F increasing	–13.5	40.0	25.6	0.5	60.0	18.5
F decreasing	–21.9	39.2	37.7	–4.6	61.7	23.8
F constant	–13.3	41.1	30.9	–2.5	59.6	21.4

[1]Bias is measured here as the relative error of the estimated value, (estimate – true) / true.
[2]The Stock Synthesis program estimates average biomass for each year of the input data series. The values here are the averages and standard deviations of the ten annual estimates.
[3]The Stock Synthesis program can estimate recruitment for each year of the input data series. The values here are the averages and standard deviations of the ten annual estimates.
[4]The "improvement in fit" is measured here by the relative increase in the value of the log-likelihood when the Stock Synthesis program estimates the annual recruitment rather than being given the true values. This is defined as

$$(L' - L) / L$$

where L is the value of the log-likelihood when the program was given the true annual recruitment values, and L' is the value of the log-likelihood when the program estimated the annual recruitment values. The log-likelihood is given by

$$\Sigma_y J_y \cdot \Sigma_a p_{y,a} \log_e(\hat{p}_{y,a});$$

where J_y is the number of fish in the (y)th catch-at-age sample, $p_{y,a}$ is the observed proportion of fish in the (y)th sample that are from the (a)th age class, and $\hat{p}_{y,a}$ is the predicted proportion of fish in the (y)th sample from the (a)th age class (Methot, 1990).

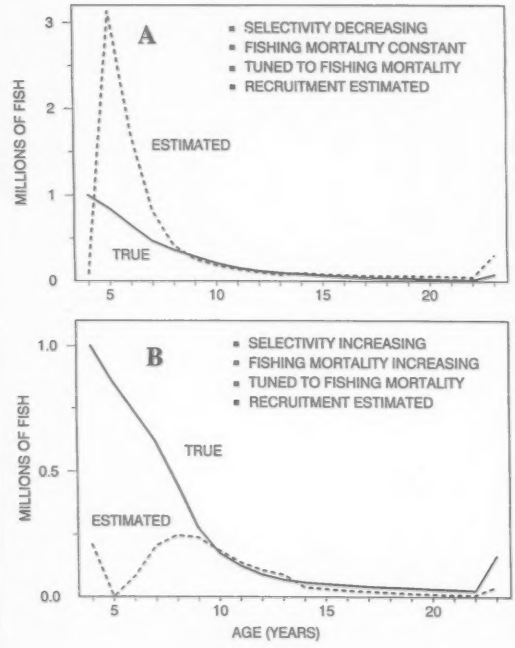


Figure 3

True versus estimated age distributions. For the age distribution shown in the upper panel (**A**) the Stock Synthesis program produced an estimate of average biomass in the final year that was 74% too large. For the distribution in the lower panel (**B**) the program produced an estimate that was 59% too small.

Sensitivity of other stock assessment programs that assume constant selectivity

The results of the experiments with CAGEAN and the multiplicative catch-at-age model indicate that these assessment methods, like the Stock Synthesis program, are also sensitive to violations of the constant selectivity assumption. When selectivity increased, both the Stock Synthesis and CAGEAN programs incorrectly produced a large decrease in the biomass of the simulated population during the last few years, and large declines in recruitment (Fig. 5). The multiplicative catch-at-age model does not provide estimates of biomass, but its estimates of relative recruitment were almost identical to those from the CAGEAN program. When selectivity decreased, the Stock Synthesis program and the CAGEAN program both overestimated the biomass in the last few years, but the estimates from the CAGEAN program were grossly incorrect (Fig. 6). Both the CAGEAN program and the multiplicative catch-at-age model estimated very large increases in recruitment.

Sensitivity of the Stock Synthesis program when applied to data from a heavily exploited stock

When the Stock Synthesis program was applied to data for a simulated population suffering heavy exploitation, the program produced estimates of biomass and recruitment that were even more biased than in the

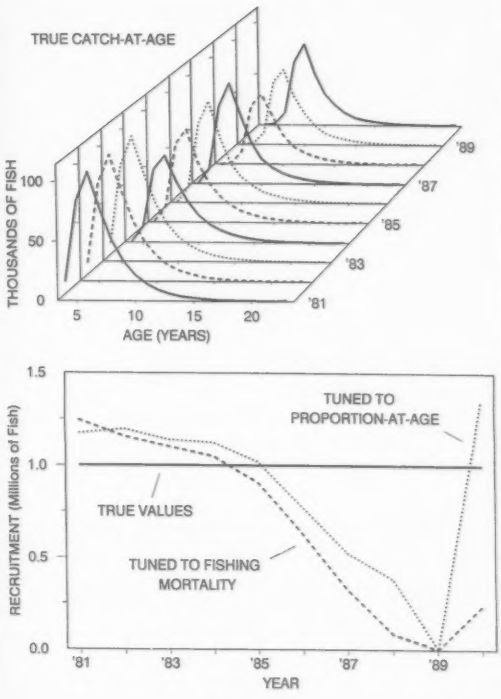


Figure 4

Trends in selectivity induce trends in recruitment. True catches-at-age (upper panel) shift through time towards older fish because of the changes in selectivity. Fishing mortality was constant for the entire period. The Stock Synthesis program attempted to mimic the changes in catch-at-age by generating estimates of recruitment that had a decreasing trend (lower panel).

earlier experiments with the simulated stock of widow rockfish. With increasing selectivity, the bias in the estimated average biomass in the final year was –86% (Fig. 7) as opposed to the bias of –54% found earlier (Table 3A; selectivity increasing, tuned to fishing mortality, fishing mortality constant, and recruitment estimated). With decreasing selectivity, the estimated average biomass in the final year was 213% too high (Fig. 8); in the earlier experiment the corresponding estimate was only 74% too high (Table 3A; selectivity decreasing, tuned to fishing mortality, fishing mortality constant, and recruitment estimated).

Discussion

In the experiments described in this paper, the assessment programs were unable to fit exactly the simulated catch-at-age data because the catch model was mis-specified. Selectivity was falsely assumed constant,

Table 4

Sensitivity analysis of the Stock Synthesis program. Bias in the estimated average biomass in the final year when selectivity varied randomly.

	Known recruitment		Estimated recruitment	
	Mean (%)	SD (%)	Mean (%)	SD (%)
Tuned to fishing mortality				
F increasing	–1.4	0.8	–19.7	21.9
F decreasing	11.3	4.4	–17.0	24.0
F constant	7.8	3.5	–18.6	23.2
Tuned to proportion-at-age				
F increasing	–0.1	0.8	–4.2	3.5
F decreasing	13.3	4.6	–8.5	5.7
F constant	8.9	3.8	–6.0	4.9

and the biomass and abundance estimates were biased as a consequence. In any real application, not only would the assessment program be ignorant of the true model structure, but the program would also have to contend with "noise" in the data due to measure-

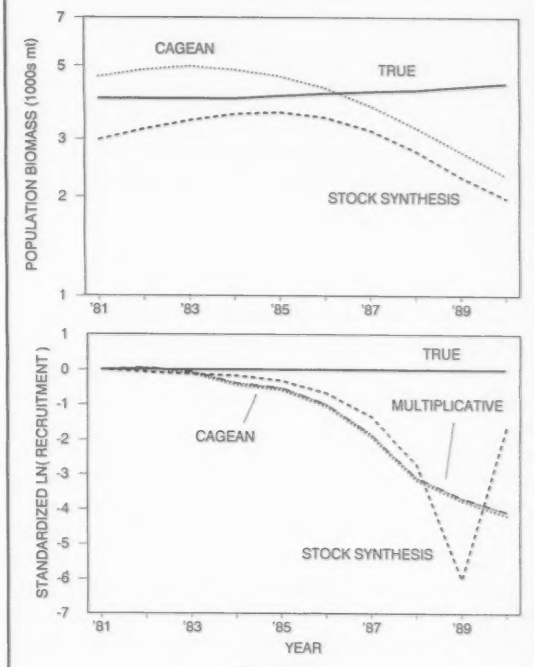


Figure 5

Estimated biomass and recruitment from different assessment programs with selectivity increasing. Three assessment programs, all of which assume constant selectivity, were applied to the simulated widow rockfish data in which selectivity shifted to older ages and fishing mortality was constant (Table 2A). All three programs were sensitive to the shifts in selectivity.

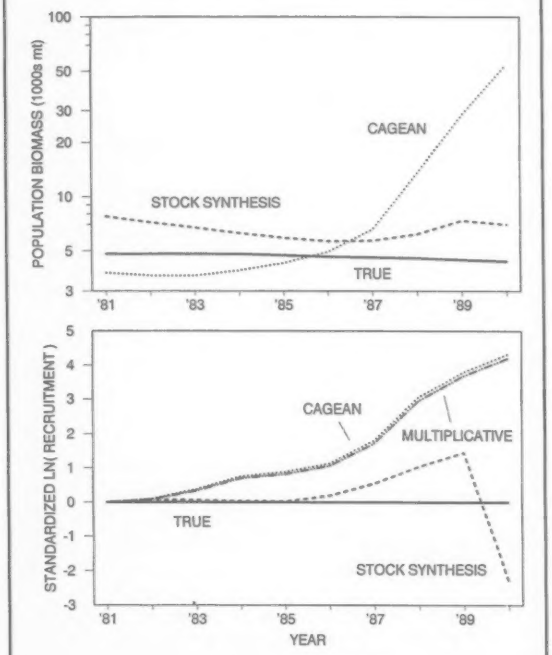


Figure 6

Estimated biomass and recruitment from different assessment programs with selectivity decreasing. The three assessment programs were also applied to the simulated widow rockfish data in which selectivity shifted to younger ages and fishing mortality was constant (Table 2B). Again, all three programs were sensitive to the shifts in selectivity.

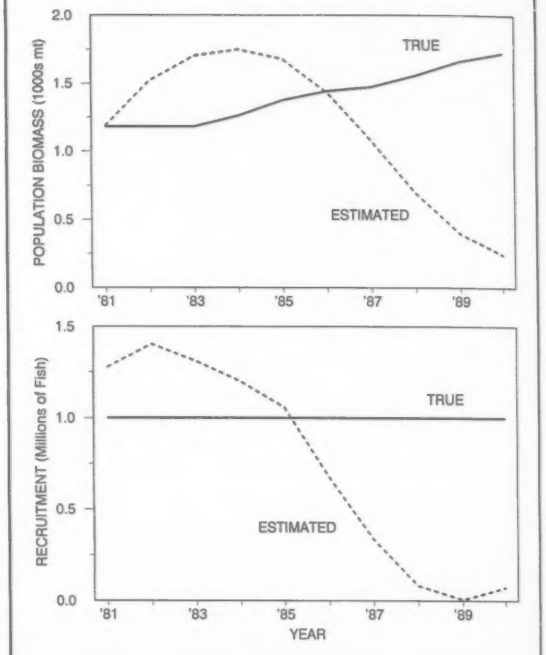


Figure 7

Stock synthesis estimates of biomass and recruitment for a heavily fished stock with selectivity increasing. The Stock Synthesis program was applied to simulated widow rockfish data in which the fishing mortality rate was constant at 0.60 per year, the natural mortality rate was 0.30 per year, and selectivity shifted to older ages. The estimates were even more biased than the ones from the corresponding earlier experiment.

ment errors and to randomness in the catch process. A complete analysis of the problem would measure how the assessment program transforms variability in the catch-at-age data into variability in the resulting estimates (e.g., Kimura, 1989).

Because the catch model with constant selectivity has fewer unknown parameters, when applied to noisy catch-at-age data, the assessment program's estimates could obtain greater precision (but not accuracy) by assuming constant selectivity, even though the assumption was incorrect[3]. However, it seems unlikely that noise in the data could ever reduce the bias resulting from a structural deficiency in the underlying catch-at-age model.

In the experiments with the simulated catch-at-age data, the year-to-year changes in selectivity were not particularly drastic, but I know of no studies to support my conjecture that they are realistic for the stock of widow rockfish. The simulated changes in selectivity were comparable to those observed by Houghton and Flatman (1981) for North Sea cod and by Gordoa and Hightower (1991) for Cape hake. The fact that experiments with "random" changes in selectivity produced results similar to those from experiments with trends in selectivity confirm that the biased estimates were not just artifacts of having a simple trend in selectivity, rather than a more complex type of variation.

One surprising result of the experiments with different assessment methods was the large discrepancy between the estimates from Stock Synthesis and CAGEAN when selectivity decreased (Fig. 6). The two programs differ primarily in how they account for vari-

[3]John Shepherd, Ministry of Agriculture, Fisheries, and Food, Fisheries Laboratory, Lowestoft, Suffolk, NR33 0HT, U.K., pers. commun. April 1992.

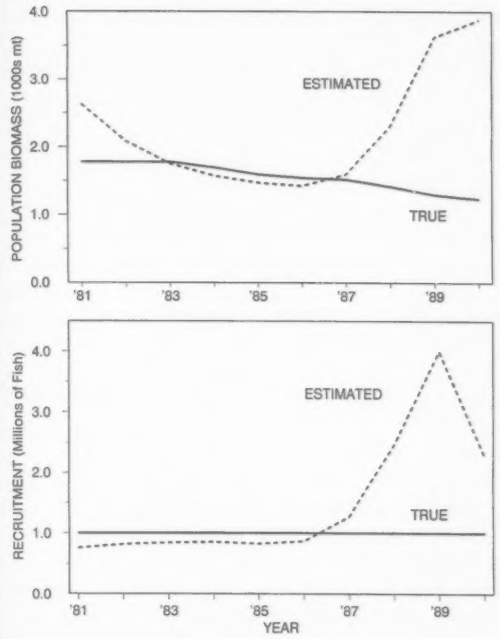


Figure 8

Stock synthesis's estimates of biomass and recruitment for a heavily fished stock with selectivity decreasing. The Stock Synthesis program was applied to simulated widow rockfish data in which the fishing mortality rate was constant at 0.60 per year, the natural mortality rate was 0.30 per year, and selectivity shifted to younger ages. Again, the estimates were more biased than the ones from the corresponding earlier experiment.

ability in catch-at-age. Stock Synthesis assumes multinomial error, but CAGEAN assumes lognormal error. Using simulation techniques, Kimura (1990) directly compared estimates derived by using these alternative assumptions and found little difference between the estimates obtained.

Another difference between Stock Synthesis and CAGEAN is in their method for modeling selectivity. The Stock Synthesis program uses a double-logistic curve to model selectivity as a smooth function of age, but the CAGEAN-PC program estimates the selectivity coefficients independently for each age. Because the true selectivity coefficients were based on double-logistic curves, this difference between the two programs should be only a minor factor. Kimura (1990) found that the assumption of a functional form for selectivity had little effect on his analyses of simulated catch-at-age data, provided the true selectivity coefficients conformed to the general shape of the selectivity function.

The dilemma for the assessment scientist is to develop a framework for analyzing fisheries data that is simple to use and yet is adequate to describe the complex dynamics of a living and constantly changing fish stock. The assessment scientist has the difficult task of interpreting diverse and possibly conflicting information. He needs tools with which to weigh these data objectively and to draw from them reliable conclusions about the status of a stock. Stock Synthesis, CAGEAN, and the multiplicative catch-at-age analysis were designed to be such tools.

In principle, one can use the Stock Synthesis and CAGEAN programs to test for shifts in selectivity. Both programs support a limited form of variable selectivity in which abrupt changes can occur at pre-specified times with constant selectivity during the intervening periods. With either program it is a relatively simple, but tedious, matter to re-analyze the data by using different times for the selectivity changes. For example, I applied the CAGEAN program to the simulated widow rockfish data set given in Table 2A, with the data partitioned into two periods of constant selectivity, and I allowed the timing of the selectivity change to occur between all possible adjacent years. The resulting pattern in the residual sums of squares[4] (Fig. 9, upper panel) clearly indicates the true change in selectivity that occurred between the third and fourth years. I repeated the process with the data series partitioned into three periods of constant selectivity, one for the first three years, and the other periods for the remaining years. The CAGEAN program was able to fit the data exactly when selectivity changed between the seventh and eighth years (Fig. 9, lower panel).

Although the current versions of the Stock Synthesis and CAGEAN programs can be applied in the above fashion to explore systematically for changes in selection, such a brute force approach to model building is extremely repetitious and time-consuming. I hope that the next generation of stock assessment programs will automate this process in a manner similar to existing stepwise regression programs, and thereby allow the user to test rigorously for variations in selectivity.

The model used for stock assessment should not force the data to fit a particular structure unless there is evidence that the structure is real or that it does not appreciably distort the results of the assessment. The

[4]CAGEAN defines the residual sum of squares as

$$\sum [\log_e(c) - \log_e(\hat{c})]^2$$

where c and $\hat{c}$ are the observed and predicted catch-at-age.

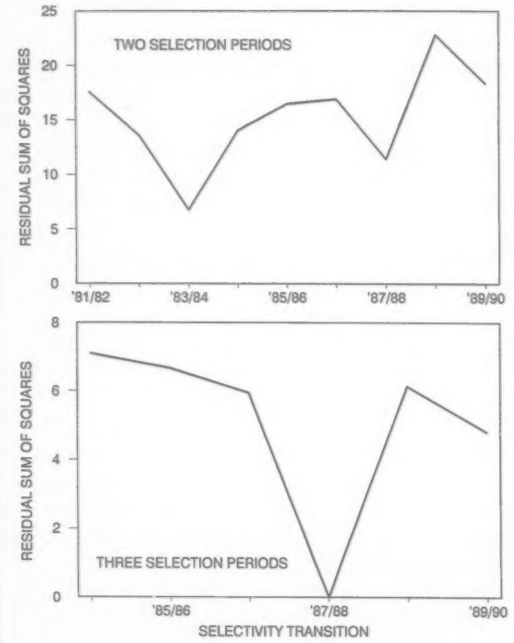


Figure 9

Residual sum of squares resulting from applications of CAGEAN with changes in selectivity. The CAGEAN program was applied to the simulated widow rockfish data in which selectivity shifted to older ages and fishing mortality was constant (Table 2A). In the upper panel the selectivity coefficients were allowed to vary abruptly between adjacent years, thereby dividing the data into two periods of constant selectivity. The minimum in the residual sum of squares corresponds to the true change in selectivity that occurred between 1983 and 1984. In the lower panel, selectivity was constant for the first three years but was allowed to vary between adjacent years in the remaining period. The zero in the residual sum of squares corresponds to the true change in selectivity that occurred between 1983 and 1984. In these analyses the age at 100% selection was fixed at age 7 for the first selectivity period, at age 8 for the second period, and at age 9 for the third.

situation is analogous to an application of two-way analysis of variance (ANOVA). In fitting a two-way ANOVA model, one should test for a significant interaction term before drawing inferences about the main effects. By the same logic, in fitting a catch-at-age model, one should test for changes in selectivity before concluding that stock size has been increasing or decreasing.

I have no real evidence of changes from year to year in the selectivity for widow rockfish off the coasts of Washington, Oregon, and California. However, the work described in this paper demonstrates that an incorrect assumption of constant selectivity can seriously distort an assessment of widow rockfish stock size. Furthermore, there is at least one reason to suspect that selectivity for widow rockfish has varied through time. During the early years of the directed fishery for widow rockfish, vessels targeted schools of fish using midwater trawls. With the rapid expansion of the fishery, the Pacific Fishery Management Council began imposing increasingly restrictive limits on the amounts of widow rockfish that could legally be landed from any single fishing trip (Gunderson, 1984). One result of these "trip limits" was a reduction in the landings by midwater trawlers relative to the landings by bottom trawlers. Midwater trawlers accounted for roughly 75% of the widow rockfish landings in Oregon during 1984 through 1988, but they accounted for only 60% in 1990, and for less than 50% in 1991. It seems quite probable that the midwater trawls have different selection characteristics than do bottom trawls, and that the shift from a midwater fishery to a bottom fishery would cause changes in selectivity.

Any stock assessment model will have to make simplifying assumptions to summarize succinctly the major features of the data. However, in my view the assumption of constant selectivity is an unnecessary and misleading oversimplification, use of which can result in catch quotas that are either needlessly conservative, resulting in immediate losses to the fishing industry, or that are excessively liberal, producing losses in recruitment and catches at a more distant time.

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Genetic analysis of the population structure of yellowfin tuna, *Thunnus albacares*, from the Pacific Ocean*

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Abstract.–The genetic basis of the population structure of yellowfin tuna, *Thunnus albacares*, in the Pacific Ocean was investigated with restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA). Samples of 20 yellowfin tuna were examined from each of five Pacific locations and one Atlantic location. MtDNA analysis with 12 informative restriction endonucleases demonstrated considerable genetic variation, as evidenced by an overall nucleon diversity of 0.84 and a mean nucleotide sequence diversity of 0.91%. Estimates of within-sample variation were remarkably consistent across all six locations. Despite high levels of variation, there was no evidence of genetic differentiation among samples. Common genotypes occurred with similar frequencies in all samples, and, with one exception, all genotypes that were represented by more than one individual occurred at more than one location. We could not reject the null hypothesis that all yellowfin tuna share a common gene pool. Our results are consistent with the alternate hypothesis that there is sufficient gene flow within the Pacific, as well as between the Atlantic and Pacific oceans, to prevent the accumulation of significant genetic differentiation.

Yellowfin tuna (*Thunnus albacares*) occur in the tropical and subtropical oceans and support major commercial fisheries throughout their range (Collette and Nauen, 1983). The economic importance of this species is indicated by high annual catches that have increased from 596,764 metric tons (t) in 1981 to 986,529 t in 1990, of which 66 to 69% were from the Pacific Ocean (FAO, 1992). Recently, purse-seine and longline fisheries in the western Pacific (120°E to about 180°) provided a major share of yellowfin tuna landings, with a catch of 342,921 t in 1990 (Lawson, 1991). In the eastern Pacific (east of 130°W) record landings near 270,000 t occurred in each of the past 3 years[1].

A thorough understanding of yellowfin tuna population structure is necessary for the effective management of this economically important, marine resource. A variety of studies, including tagging, morphometric, fishery statistic and genetic analyses, have been used to infer population structure. However, the proposed population structures differed.

Tagging studies have indicated that movements of yellowfin tuna in the Pacific Ocean tend to be geographically restricted. Fink and Bayliff (1970) analyzed tag return data in the eastern Pacific and proposed a northern and southern group of fish with some exchange between groups. There was very limited westward movement of tagged fish reported in the study; however, as the eastern Pacific fishery expanded westward in subsequent years, several returns were obtained from farther offshore indicating possible mixing between eastern and central Pacific fish (Bayliff, 1984). In the western Pacific tagging studies showed that most individuals remained within the western Pacific region and did not make extensive movements (Itano and Williams, 1992; Lewis, 1992). Although the majority of recaptured yellowfin tuna in all studies showed limited movement, some returns were obtained which demonstrated the potential for fish to move large dis-

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Fishery Bulletin 91:690–698 (1993).

[1]Anonymous. 1992. Inter-American Tropical Tuna commission, Annual Report, 1991.

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tances and between regions (Fink and Bayliff, 1970; Bayliff, 1984; Itano and Williams, 1992).

Population structure was indicated by investigations of both meristic and morphometric characters which revealed significant differentiation among yellowfin tuna from the eastern, central, and western Pacific regions (Schaefer, 1955; Kurogane and Hiyama, 1957), as well as clinal character variation across the equatorial Pacific (Royce, 1964). Further investigation using discriminant function analysis of morphometric variables suggested mixing occurs between morphologically differentiated northern and southern yellowfin tuna of the eastern Pacific (Schaefer, 1989), as well as across the Pacific (Schaefer, 1991, 1992).

Analysis of fishery data also suggests population structuring of yellowfin tuna within the Pacific. Kamimura and Honma (1963) provided evidence for two or more semi-independent subpopulations based on size composition and catch data of equatorial Pacific yellowfin tuna from longline landings. Suzuki et al. (1978) examined longline and purse-seine length composition data and suggested the existence of semi-independent eastern, central, and western Pacific subpopulations. Additionally, homogeneity within the western Pacific was indicated by the widespread distribution of fish contaminated by radioactivity resulting from the 1954 U.S. nuclear tests at Bikini Atoll (Suzuki et al., 1978).

While the results of several analyses suggest yellowfin tuna exhibit population structure within the Pacific Ocean, genetic analyses have revealed no significant genetic differentiation. Suzuki (1962) reported that the blood agglutinogen Tg2 occurs in similar frequencies in samples from the Indian Ocean and the eastern Pacific. Additionally, allozyme analysis did not reveal genetic differentiation between samples of yellowfin tuna collected off Hawaii (n=529) and Baja California (n=207) at the polymorphic serum esterase locus, although overall variation was low (Fujino, 1970). Preliminary evidence for frequency differences occurring at two other loci (phosphoglucose isomerase and transferrin A) was reported for both within and between samples of Atlantic and Pacific yellowfin tuna[2,3]. However these loci have not been used to examine population structure.

Our understanding of the population structure of yellowfin tuna in the Pacific Ocean remains problematic. Much evidence is available which suggests that population structure exists, yet genetic analyses did not reveal differentiation among samples collected from distant locations. To further examine the genetic basis of the population structure of Pacific yellowfin tuna we employed restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA). Because mtDNA evolves rapidly (Moritz et al., 1987; Brown et al., 1979) and displays considerable polymorphism within animal populations (Avise and Lansman, 1983), mtDNA analyses have been useful in revealing population structure within marine fishes (Ovenden, 1990). Using this technique, we demonstrated considerable genetic variability within yellowfin tuna, but we could not reject the null hypothesis that samples share a common gene pool.

Materials and methods

Hearts were taken from 50 yellowfin tuna at each of five Pacific locations and one Atlantic location (Fig. 1); however, only 20 specimens per location were analyzed. Samples from the Pacific were collected during 1990 at Manta, Ecuador (ECU); Revillagigedo Islands, Mexico (MEX); Oahu, Hawaii (HAW); Manus Island, Papua New Guinea (PNG); and New South Wales, Australia (AUS). The sample from the Atlantic was collected during 1991 at Hatteras, North Carolina (ATL). Hearts were dissected within 12 hours of capture and placed on crushed ice. Hearts from fish collected in the Pacific were frozen at –20°C and shipped to the Inter-American Tropical Tuna Commission, La Jolla, CA, where they were stored at –20°C for more than one year before shipment to our laboratory. Hearts from fish collected in the Atlantic were transported on wet ice and frozen at –70°C within four hours of dissection.

MtDNA was purified from 3 g of heart tissue from Atlantic specimens following the CsCl-ethidium bromide gradient centrifugation protocol of Lansman et al. (1981). MtDNA yields averaged about 350 ng of supercoiled mtDNA per g of heart tissue. Aliquots of mtDNA were digested with the following 12 informative restriction endonucleases (Stratagene and BRL) according to the manufacturers' instructions: *Apa*I, *Ava*I, *Ban*I, *Bcl*I, *Bgl*I, *Dra*I, *Eco*RI, *Hin*dIII, *Nci*I, *Pst*I, *Pvu*II and *Xho*I. Restriction fragments were end-labeled with a mixture of all four α-^{35}S-dNTP's by using the Klenow fragment, electrophoresed at 2 volts/cm overnight in 1% agarose gels, and visualized by autoradiography (Sambrook et al., 1989).

Yields of supercoiled mtDNA from Pacific specimens were low, possibly due to sub-optimal storage conditions. For these specimens, mtDNA-enriched genomic DNA was isolated from 4 to 6 g of heart tissue following the protocols of Chapman and Powers (1984), modified by the omission of sucrose step gradients and the

[2]Anonymous. 1977. Inter-American Tropical Tuna Commission, Annual Report, 1976.

[3]Anonymous. 1978. Inter-American Tropical Tuna Commission, Annual Report, 1977.

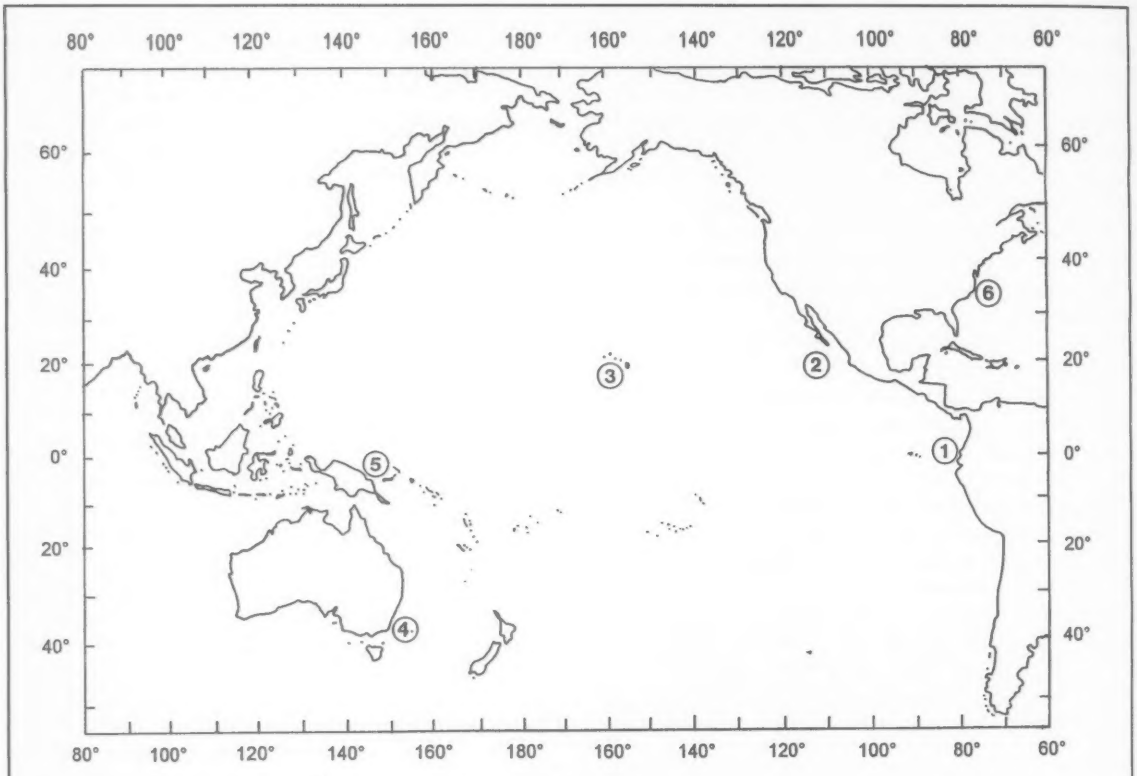

Figure 1

Thunnus albacares. Location of sampling sites used in this analysis: (1) Manta, Ecuador (ECU); (2) the Revillagigedo Islands, Mexico (MEX); (3) Oahu, Hawaii (HAW); (4) New South Wales, Australia (AUS); (5) Manus Island, Papua New Guinea (PNG); and (6) Hatteras, North Carolina (ATL).

use of 1.5% sodium dodecyl sulfate for mitochondrial lysis. Following restriction enzyme digestion and horizontal agarose gel electrophoresis, DNA fragments were transferred to nylon membranes by Southern transfer (Sambrook et al., 1989) and immobilized by long-wave UV irradiation. Prehybridization was conducted for two hours at 42°C in 50% formamide, 5× SSC, 5× Dendhardt's solution, 0.025 mM $NaPO_4$, pH 6.5, and 100 µg/mL heat denatured calf thymus DNA. Probe DNA (mtDNA purified from extra specimens from the Atlantic) was nick-translated to incorporate biotin-7-dATP (BRL), and separated from unincorporated nucleotides by size exclusion chromatography. One µg of probe was added to the prehybridization solution for each 200 cm^2 blot and allowed to hybridize overnight at 42°C. Following post-hybridization washes (Sambrook et al., 1989), mtDNA fragments were visualized with the BRL BluGene Non-Radioactive Nucleic Acid Detection Kit.

A 12-letter composite mtDNA genotype, indicating the fragment pattern for each restriction enzyme, was developed for each individual. Estimates of nucleon diversity (h) for each sample and for the pooled samples were computed following Nei (1987). Nucleotide sequence divergences among genotypes were estimated by using the site method of Nei and Li (1979). Estimates of nucleotide sequence diversity (p) within each sample, and mean nucleotide sequence divergences among samples (corrected for within-sample diversities) were computed following Nei (1987). Nucleotide sequence divergences were clustered by the unweighted pair-group method with arithmetic means (UPGMA) by using the average linkage algorithm of the SPSS-X statistical package (Norusis, 1988). Values of G_{st}, a measure of heterogeneity between samples, were estimated from sample genotype frequencies (Nei, 1987), and values of N_em, the absolute number of migrants between samples, were determined from the relation

$N_em = (1/G_{st} - 1)/2$ (Birky et al., 1983; Nei, 1987). Chi-square analysis was conducted by using the Monte-Carlo method of Roff and Bentzen (1989) with 1000 randomizations of the data to evaluate heterogeneity of genotype frequencies among samples without combining rare genotypes.

Results

Analysis of mtDNA from 120 yellowfin tuna with 12 restriction enzymes revealed a total of 34 genotypes, comprising 83 unique fragments. The most common genotype consisted of 52 fragments, representing a survey of 304 bp, or about 1.8% of the mtDNA genome. The mean size of the yellowfin tuna mtDNA genome, determined from the most common restriction fragment profiles for each of the 12 restriction enzymes, was 16,549 ± 309 bp (SD).

RFLP analysis of yellowfin tuna mtDNA demonstrated considerable variation. While four restriction enzymes, *Ava*I, *Dra*I, *Hin*dIII, and *Xho*I, showed no variation, the remaining eight revealed two to seven restriction morphs each. Of the 34 genotypes, two were represented by 20 or more individuals, five were represented by four or more individuals, and 20 genotypes occurred only once (Table 1). Within-sample nucleon diversities were high and showed little variation among samples, ranging from 0.82 to 0.86, with a value of 0.84 for the pooled samples (Table 2). Mean nucleotide sequence diversities were also high, ranging

Table 1

Distributions of mtDNA genotypes among samples of yellowfin tuna, *Thunnus albacares*. Letters represent fragment patterns produced by the following enzymes (left to right): *Eco*RI, *Hin*dIII, *Pst*I, *Dra*I, *Ava*I, *Pvu*II, *Nci*I, *Ban*I, *Bcl*I, *Bgl*I, *Xho*I, and *Apa*I. A description of fragment sizes is available upon request.

	mtDNA genotype	Sampling location						
		ECU	MEX	HAW	AUS	PNG	ATL	Total
1	AAAAAAAAAAAA	7	6	7	8	8	7	43
2	BAAAAAAAAAAA	2	5	3	4	3	3	20
3	BAAAAABAAAAA	3	—	1	—	1	1	6
4	AAAAAAAAECAA	1	1	—	2	—	2	6
5	AAAAAAAABAAA	—	—	—	1	1	2	4
6	AAAAAABAEAAA	1	—	1	—	—	1	3
7	AAAAAABABAAA	2	1	—	—	—	—	3
8	AABAAAAAAAAA	—	2	—	1	—	—	3
9	AAAAAAABAAAA	—	—	1	—	1	—	2
10	AAAAAAAABBAA	—	—	1	1	—	—	2
11	BAAAAAAAAAAB	—	—	1	—	—	1	2
12	BAAAAAAACAAA	—	—	1	—	1	—	2
13	AAAAAAAAEAAF	—	1	—	—	1	—	2
14	AAAAAABAAAAA	—	—	2	—	—	—	2
15	AAAAAAAAAAAB	—	—	—	1	—	—	1
16	AAAAABAAAAAA	—	—	—	—	—	1	1
17	AAAAAAAAAAAC	—	—	—	—	1	—	1
18	AAAAAAAAAAAD	—	—	—	—	1	—	1
19	AAAAAAAAFAAA	—	—	—	—	1	—	1
20	AAAAAAAAAAAF	—	—	1	—	—	—	1
21	AABAAAAABAAA	—	—	—	—	—	1	1
22	BABAAAAAAAAA	—	—	1	—	—	—	1
23	AAAAAABAAAAE	—	—	—	1	—	—	1
24	AAAAAAAADAAB	—	—	—	—	1	—	1
25	CAAAAABAAAAA	1	—	—	—	—	—	1
26	AABAAAAAAAAG	1	—	—	—	—	—	1
27	AABAAAAAEAAA	1	—	—	—	—	—	1
28	AAAAAABAAAAB	—	1	—	—	—	—	1
29	BAAAAAAAAAAD	—	1	—	—	—	—	1
30	BAAAAAAABBAA	—	1	—	—	—	—	1
31	BAAAAABABAAA	—	1	—	—	—	—	1
32	BAAAAABAGAAA	1	—	—	—	—	—	1
33	AAAAAABAADAA	—	—	—	—	—	1	1
34	AAAAAAACFEAA	—	—	—	1	—	—	1
Total		20	20	20	20	20	20	120

Table 2

Genetic variation within samples of yellowfin tuna, *Thunnus albacares*, expressed as nucleon diversity (h) and percent nucleotide sequence diversity (p).

Sampling location	Nucleon diversity (h)	Nucleotide sequence diversity (p) %
ECU	0.863	1.04
MEX	0.863	0.955
HAW	0.868	0.799
AUS	0.816	0.905
PNG	0.837	0.737
ATL	0.863	0.917
Pooled	0.840	0.907

from 0.74% to 1.04%, and 1.04% for the pooled samples (Table 2).

Despite the high level of within-sample variation, there was little evidence for genetic differentiation among the six sampling sites. The two most common genotypes (1 and 2) were observed at all locations at similar frequencies (30–40%, 10–25%, respectively; Table 1). Of the 12 other genotypes that occurred in more than a single individual, 11 were found in two or more samples, and only one (genotype 14) occurred in a single sample, represented by two individuals.

Pairwise estimates of corrected nucleotide sequence divergences between samples (including the Atlantic) were low, ranging from 0.012% to 0.104%, with a mean pairwise divergence of 0.04%. Similarly, estimates of G_{st} were low, ranging from 0.011 to 0.025, and values of N_fm were correspondingly high, ranging from 19.5 to 44.5 females per generation. No clear pattern of phylogeographic structure was revealed in cluster analyses of nucleotide sequence divergences among mtDNA genotypes or sampling locations. Chi-square analysis of heterogeneity among samples was not significant, as randomizations of the data into six samples were more heterogeneous than the observed genotypic distributions 581 of 1000 times (P=0.581).

To determine an appropriate number of individuals to examine from each location and the number of restriction enzymes to employ, we compared levels of genetic variation and differentiation revealed by a pilot study of 12 individuals per location with 5 enzymes with larger analyses of up to 20 individuals per location with 12 enzymes (Table 3). Levels of variation were influenced more by increasing the number of enzymes surveyed than the number of individuals. Increasing the number of enzymes increased the number of genotypes, the nucleon diversities, and, to a lesser extent, nucleotide sequence diversities. Analysis of a greater number of individuals per location had little effect on diversity estimates with either 5 or 12 enzymes, although the ranges of within-sample diversities among locations decreased as more individuals were analyzed.

Increasing the number of individuals or the number of enzymes had little effect on levels of genetic differentiation. No significant differences were found in the distributions of genotypes among locations in Roff and Bentzen (1989) chi-square tests (Table 3). Furthermore, increasing the number of individuals in the 12 enzyme analysis did not increase the frequencies of genotypes unique to a location. Instead, many unique genotypes in the analysis of 12 individuals occurred in other locations as a greater number of individuals were used. Because increasing sample sizes from 12 to 20 individuals did not reveal greater spatial partitioning of genetic variation, we decided that 20 individuals was an appropriate sample size.

Discussion

Population structure is typically manifested as the spatial or temporal partitioning of genetic variation. Therefore, to demonstrate if population structure exists, a technique must first reveal a reasonable level of genetic variation. RFLP analysis of mtDNA revealed considerable genetic variation in yellowfin tuna. The overall nucleon diversity and mean nucleotide sequence diversity (0.84 and 0.91%, respectively) are in the up-

Table 3

Pooled percent nucleotide sequence diversities (p) and nucleon diversities (h), and respective ranges (in parentheses) among samples obtained by varying the numbers of restriction enzymes and individuals of yellowfin tuna, *Thunnus albacares*, per sampling location. Probabilities of significance result from Roff and Bentzen's (1989) chi-square analysis with 1000 randomizations of the data. The five enzymes selected for the pilot study were *Eco*RI, *Hin*dIII, *Pst*I, *Dra*I, and *Ava*I.

Design	Number of genotypes	Nucleon diversity (h)	Nucleotide sequence diversity (p) (%)	Prob.
5 enzymes 12 individuals	6	0.496 (0.41–0.64)	0.705 (0.49–0.90)	0.960
5 enzymes 20 individuals	6	0.509 (0.42–0.63)	0.714 (0.62–0.91)	0.845
12 enzymes 12 individuals	27	0.829 (0.76–0.94)	0.907 (0.66–1.22)	0.975
12 enzymes 20 individuals	34	0.840 (0.82–0.87)	0.907 (0.74–1.04)	0.581

per ranges reported for marine fishes (Avise et al., 1989; Ovenden, 1990; Gold and Richardson, 1991) including other large, pelagic fishes (Table 4).

Genetic variability in tuna species has also been demonstrated by sequence analysis of a 307-base-pair region of the mitochondrial cytochrome *b* gene of bluefin tuna, *Thunnus thynnus*; bigeye tuna, *T. obesus*; albacore, *T. alalunga*; and yellowfin tuna (Bartlett and Davidson, 1991). The sequence data presented for 33 yellowfin tuna result in a nucleon diversity of $h = 0.28$, a value that is considerably lower than the nucleon diversity of 0.84 obtained in our study. Although nucleon diversity values are greatly influenced by the number of base pairs surveyed (Nei, 1987), the number examined in this study and in Bartlett and Davidson (1991) were very close (304 and 307, respectively). This difference in nucleotide sequence diversity may reflect a slower evolutionary rate for the mitochondrial cytochrome *b* gene relative to the entire mtDNA genome.

Significant genetic differentiation among yellowfin tuna from geographically distant locations was not found. Corrected nucleotide sequence divergences averaged only 0.04%, indicating that the mean difference between two genotypes randomly chosen from any two samples was essentially the same as the difference between two genotypes randomly drawn from the same sample. The frequencies of the two most common genotypes were similar among all locations, and an overall chi-square test for heterogeneity was non-significant. Furthermore, nucleon diversities and nucleotide sequence diversities were similar among locations, although uniformity in the latter estimates was less pronounced.

Table 4

Genetic variation within selected pelagic species determined by RFLP analysis of mtDNA employing 11–13 informative enzymes. Variation is expressed as nucleon diversity (h) and percent nucleotide sequence diversity (p).

Species	Nucleon diversity (h)	Nucleotide sequence diversity (p) (%)
Thunnus albacares[1] (Yellowfin Tuna)	0.84	0.91
Thunnus alalunga[2] (Albacore Tuna)	0.60	0.31
Tetrapturus audax[3] (Striped Marlin)	0.74	0.54
Tetrapturus albidus[3] (White Marlin)	0.70	0.35
Makaira nigricans[3] (Blue Marlin)	0.86	1.99
Istiophorus platypterus[3] (Sailfish)	0.62	0.87

[1]This study.
[2]Graves and Dizon (1989) and Graves, unpublished data.
[3]Graves and McDowell, in press.

The apparent genetic homogeneity of yellowfin tuna is consistent with the hypothesis that there is genetic exchange among locations. Rare mtDNA genotypes had low frequencies of occurrence (Table 1), characteristic of high gene flow (Slatkin, 1985), a situation similar to that found in other species for which high gene flow is suggested: American eel, *Anguilla rostrata* (Avise et al., 1986); marine catfishes, Ariidae (Avise et al., 1987); weakfish, *Cynoscion regalis* (Graves et al., 1992a); and bluefish, *Pomatomus saltatrix* (Graves et al., 1992b). Low pairwise estimates of G_{st} (0.011–0.025) also indicated homogeneity in yellowfin tuna and gave rise to high N_em values (>19) which are consistent with high rates of gene flow among regions (Birky et al., 1983).

The absence of genetic differentiation between Pacific and Atlantic samples of yellowfin tuna is similar to that reported for other vagile pelagic species. RFLP analysis of mtDNA of skipjack tuna, *Katsuwonus pelamis*, and of albacore from the Atlantic and Pacific oceans revealed modest amounts of within-sample variation, but no significant differentiation was found between Atlantic and Pacific conspecifics (Graves et al., 1984; Graves and Dizon, 1989). Similarly, RFLP analysis of mtDNA of the pelagic dolphin, *Coryphaena hippurus*, revealed no significant differentiation between samples from the Atlantic and Pacific oceans[4]. However, spatial partitioning of genetic variation occurs in at least some pelagic fishes. Significant genetic differentiation was shown between Atlantic and Pacific blue marlin, *Makaira nigricans*, by direct sequence analysis of the mitochondrial cytochrome *b* gene (Finnerty and Block, 1992) and RFLP analysis of mtDNA (Graves and McDowell, in press). Similarly, differentiation was found among striped marlin samples, *Tetrapturus audax*, and sailfish, *Istiophorus platypterus*, from the Pacific Ocean by RFLP analysis of mtDNA (Graves and McDowell, in press).

There are several characteristics of yellowfin tuna which could promote gene flow among locations. Yellowfin tuna are distributed circumtropically (Collette and Nauen, 1983) and occur around the Cape of Good Hope in the southern summer (Talbot and Penrith, 1962). Tagging studies demonstrated that adults are capable of traveling large distances between Pacific regions (Fink and Bayliff, 1970; Bayliff, 1984; Itano and Williams, 1992), and are capable of undergoing trans-Atlantic crossings (Bard and Scott, 1991). The existence of suitable spawning areas throughout the

[4]Carol Reeb, University of Hawaii, pers. commun. April 1992.

tropical oceans, suggested by circumtropical occurrence of larvae (Nishikawa et al., 1985), would permit unobstructed gene flow throughout the species' distribution.

Both genetic and morphological analyses revealed considerable variation in yellowfin tuna; however, genetic analyses indicated that differentiation does not occur. Evidence that morphological characters are environmentally influenced was provided by comparison of our data to the morphological differentiation found in Schaefer (1991, 1992). In our analysis, 20 fish from four locations (Ecuador, Mexico, Hawaii, and Australia) were the same as those used in Schaefer's studies. Although no genetic differences were found among these locations, morphometric characters and gill-raker counts differed significantly, which we conclude is the result of phenotypic plasticity. The finding of greater morphological variability among Pacific samples than occurred between the Atlantic and Pacific (Schaefer and Walford, 1950) also supports the hypothesis that phenotypic plasticity is the cause of morphological differentiation in the Pacific.

The null hypothesis that yellowfin tuna in the Pacific Ocean share a common gene pool could not be rejected in this analysis. Our results were consistent with the alternate hypothesis that yellowfin tuna maintain sufficient gene flow among areas to prevent the accumulation of significant genetic differentiation. As theoretical models indicate that very low levels of migration (only a few individuals per generation) are needed to prevent genetic differentiation among large populations (Allendorf and Phelps, 1981; Hartl and Clark, 1989), the present data indicate only that some minimal amount of exchange is occurring. Other approaches are necessary to quantify the amounts of mixing among regions and to judge more accurately whether or not separate stocks should be distinguished for management purposes.

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Planktonic and benthic feeding by the reef-associated vermilion snapper, *Rhomboplites aurorubens* (Teleostei, Lutjanidae)*

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Abstract.–The feeding habits of the vermilion snapper, *Rhomboplites aurorubens*, were investigated to determine the role of this ecologically dominant and economically valuable species in the trophic ecology of southeastern U.S. reef habitats. Trophic ecology was studied by examining stomach contents and by comparing them to samples of benthic invertebrates and near-bottom plankton. Vermilion snapper fed on a variety of infaunal, epifaunal, and pelagic invertebrates, as well as on demersal and pelagic fishes and cephalopods. The diet was diverse but was dominated numerically by planktonic species. The benthic prey eaten were species associated with hard-bottom reef structure or were infaunal species from sand bottom areas adjacent to the reef. Many species were members of the hyperbenthos or demersal zooplankton and were apparently consumed in the water column during their nocturnal emergence from the sand or reef. Small crustaceans, especially copepods, sergestid decapods, and larvae of barnacles, stomatopods and decapods, dominated the diet of small (<50 mm SL) vermilion snapper. Larger decapods, fishes, and cephalopods were more important in the diet of larger vermilion snapper. Vermilion snapper, although reef associated, does not feed heavily on reef species, and may be important in transferring energy from the water column and adjacent sandy areas to the reef.

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The vermilion snapper, *Rhomboplites aurorubens*, is the most abundant lutjanid in the recreational and commercial fisheries of the southeastern U.S. (Grimes et al., 1982), and is a dominant component of the reef-associated ichthyofauna off the Carolinas and Georgia (Sedberry and Van Dolah, 1984). Because of its abundance and habit of foraging on small pelagic crustaceans (Grimes, 1979), vermilion snapper may be an important trophic link between the water column and those reef habitats where it schools during resting periods. If vermilion snapper also forages on infaunal benthos in adjacent sandy areas, it may be very important in the trophic coupling between the reef and surrounding expansive sandy areas. Vermilion snapper is also fed on by other predatory fishes (Sedberry, 1988) and thus may provide trophic links among top-level carnivores and the infauna, holozooplankton, demersal zooplankton (*sensu* Alldredge and King, 1977), and reef benthos.

Predation by fishes has been shown to be important in transferring energy from the water column or adjacent sand bottom areas to reef habitats (Bray et al., 1981; Meyer and Schultz, 1985; Rothans and Miller, 1991). Although information on feeding is available for some species of reef-associated fishes off the southeastern United States (Manooch, 1977; Grimes, 1979; Sedberry, 1985, 1987, 1988), the importance of reef bottom versus adjacent water column and sand bottom habitats as feeding grounds is poorly understood. These reefs support not only a variety of large sessile invertebrates (e.g., sponges, corals, tunicates) and associated motile organisms (Struhsaker, 1969; Wenner et al., 1983) but also a greater faunal abundance, diversity, and biomass than adjacent sand bottom areas of the open shelf (Struhsaker, 1969; Wenner, 1983; Wenner et al., 1983). Because of the rocky outcrops and the warming influence of the Florida Current, these reefs support tropical and subtropical families of fishes, and many economically valuable serranids, haemulids, sparids, and lutjanids, including vermilion snapper (Miller and Richards, 1980; Chester et al., 1984). The greater biomass and diversity of rocky reef habitats, compared with

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sandy areas, may be the result of trophic links through reef-asssociated fishes, such as vermilion snapper, with other ecotopes on the shelf.

While an important component of regional reef ecosystems and fisheries, vermilion snapper and demersal-feeding fishes, such as red porgy (*Pagrus pagrus*) are being overfished (Low et al., 1985; Collins and Sedberry, 1991). As a result of this increased fishing pressure, vermilion snapper has increased in abundance relative to *P. pagrus* and other overexploited reef fishes off the southeastern United States, and the importance of its functional role in reef ecosystems may have changed. Increased fishing pressure since the late 1970's has also apparently caused a concomitant decrease in mean length and a decrease in size at maturity of vermilion snapper (Collins and Pinckney, 1988; Collins and Sedberry, 1991). If small vermilion snapper consume different prey than do large vermilion snapper and if average fish size is decreasing in the region, the trophic structure of reefs may be affected.

The objectives of this study were to describe the feeding habits of vermilion snapper and to evaluate its relative dependence on hard-bottom benthos, sand-bottom infauna, demersal zooplankton (Alldredge and King, 1977, 1985; Porter and Porter, 1977), holozooplankton (Cahoon and Tronzo, 1992) and nekton as food. An additional purpose was to describe differences in feeding habits with size.

Methods

Stomachs from vermilion snapper were collected during six cruises in 1980 and 1981 from 11 reef stations off South Carolina and Georgia. Stations were located in each of three depth zones representing the inner shelf (16–22 m depth, three stations), middle shelf (23–37 m depth, four stations) and outer shelf (46–69 m depth, four stations). Delineation of depth zones was based on distribution of fish assemblages as noted in previous studies and on community analysis of catches in the present study (Struhsaker, 1969; Miller and Richards, 1980; Sedberry and Van Dolah, 1984). Fishes were collected primarily with a roller-rigged 40/54 high rise trawl (Hillier, 1974); a few were captured with traps or hook-and-line. Sampling was conducted during the day and at night on hard-bottom reef habitat that was mapped for each station by means of underwater television. Detailed descriptions of station locations and fish sampling techniques have been described elsewhere (Sedberry and Van Dolah, 1984; Wenner et al., 1984).

Standard lengths (SL in mm) were measured at sea. Stomachs were removed, individually labeled, and fixed in 10% seawater-formalin. Because of limited space and very large catches, only those stomachs that appeared to contain ingesta were preserved. No attempt was made in the field to determine the percentage of stomachs with food. Small (<50 mm SL) vermilion snapper were preserved whole and dissected in the laboratory. Fixed stomachs and small individuals were washed in tap water and transferred to 50% isopropyl alcohol.

Contents of individual stomachs were sorted by taxa and counted. Volume displacement of food items was measured in a graduated cylinder or estimated by using a 1×1 mm grid (Windell, 1971). The relative contribution of food items to the diet was determined by using three methods:

$$\text{percent frequency occurrence, } F = \frac{\text{number of stomachs with prey taxon}}{\text{number of stomachs with food}} \times 100$$

$$\text{percent numerical abundance, } N = \frac{\text{number of individuals of prey taxon}}{\text{total number of prey items}} \times 100$$

$$\text{percent volume displacement, } V = \frac{\text{volume displacement of prey taxon}}{\text{total volume of all prey items}} \times 100 .$$

These values are presented only for those prey species that occurred with a frequency of at least one percent or that made up at least one percent of the total number or volume of prey. Values of F, N, and V were also calculated for higher prey taxa, for stomachs pooled by 50-mm intervals of SL of vermilion snapper. The chi-square statistic (Tyler, 1979) was used to test for significance (0.05 level) of feeding heterogeneity between predator size classes.

To determine selection of prey type and predator feeding habitat, stomach samples were compared to samples from the potential prey environment by using Ivlev's index of electivity (Ivlev, 1961), calculated as

$$E = \frac{r_i - p_i}{r_i + p_i} ,$$

where: E = electivity for the ith potential prey species; r_i = percent by number of species i in the diet; and p_i = percent by number of species i in samples from the environment. Electivity values range from −1 to +1. Negative values imply that the species is avoided, not preferred, only incidentally ingested, or unavailable to the predator. Positive values imply that the predator prefers the prey species or that it is feeding on prey species that occur in a different habitat than that sampled by the prey sampler. A value near zero im-

plies no selectivity by the predator, that is to say the fish is feeding on the prey in proportion to the prey's relative abundance in samples taken in the habitat.

The electivity index was calculated for species that were numerically dominant in prey environment samples or in fish stomach samples. Dominant species included those that ranked among the five most abundant species within stomach or environmental samples pooled by depth zone. Prey samples and stomach collections were pooled by depth zone (inner, middle, and outer shelf) for comparison. Samples of benthic prey were obtained at the 11 reef sites during 1980 and 1981 with a diver-operated suction sampler (inner and middle shelf) or a grab sampler (outer shelf). Details of benthic sampling are provided elsewhere (Wenner et al., 1983; Wenner et al., 1984) and are only summarized here. Briefly, divers obtained five replicate suction samples during the day at each inner and middle shelf reef site by scraping the hard reef substrate enclosed by a 0.1-m^2 quadrat box while simultaneously sucking with an airlift device similar to that described by Chess (1979). Suction samples were collected in 1.0-mm mesh bags.

At the outer shelf stations, where water depth precluded the use of the suction device operated by divers, quantitative 0.1 m^2-samples were collected in reef habitat with a modified Smith-McIntyre grab. After retrieval, each sample was sieved through a 1.0-mm sieve and retained organisms were identified and counted. Most (75.0%) grab samples were taken during the day.

Additional sampling was conducted to compare stomach contents with potential prey species occurring in the near-bottom water column above the reef. To determine extent of feeding on the near-bottom plankton, electivity values were calculated for samples obtained with an epibenthic plankton sled and compared with those from the suction and grab samples. The sled was similar to that described by Brattegard and Fosså (1991) but had a mouth opening of 0.5 m^2 and runners that permitted it to sample 0.5 m off the bottom. The sled also had a mouth-opening mechanism designed to fish only when it was in contact with the bottom. A 0.947-mm mesh net was attached to the sled and two ten-minute tows were made per station at night to minimize net avoidance. Sled collections for invertebrates were made only in 1981. These samples were analyzed for decapods, stomatopods, cumaceans, and mysids, and comparisons with fish stomachs were limited to these taxa. Underwater television and diver observations were used to direct invertebrate sampling to reef habitat. Further details of all fish and invertebrate sampling can be found in Sedberry and Van Dolah (1984) and Wenner et. al. (1984).

Daytime submersible observations of vermilion snapper behavior were made during dives aimed at visually censusing (transects and point counts) reef fishes of the outer shelf on 14–15 July 1985. The submersible used was the *Sea Link I*, which provided a panoramic view (Askew, 1985) of shelf edge reefs and their fishes.

Results

Vermilion snapper was very abundant in trawl catches. Although it was relatively infrequent at inner shelf stations (mean catch per tow of 12.4), vermilion snapper was very abundant at middle (243.7 per tow) and outer shelf (140.1 per tow) stations.

Approximately 115 species of prey were identified in 255 stomachs that contained food. Fish with stomachs containing food were found at all times of the day; 22% of stomachs with food were collected between 0001 and 0600 hours local time; 17%, between 0601 and 1200 hours; 18%, between 1201 and 1800 hours; and 43%, between 1801 and 2400 hours. Although no attempt was made in the field to quantify proportions of stomachs with food, there were more stomachs with obvious contents at night (1801–0600 hr).

Most prey items found in vermilion snapper stomachs were planktonic or nektonic organisms (Table 1). Amphipods, mainly planktonic hyperiids and caprellids (e.g., *Lestrigonus bengalensis*, *Phtisica marina*), copepods, and decapods (e.g., larval forms, *Lucifer faxoni*) were the most frequently consumed taxa. These small crustaceans were eaten in large numbers by smaller vermilion snapper, but, with the exception of decapods, contributed little to prey volume (Table 2). Mysids, cumaceans, and doliolids were also frequently consumed. The overall diet was dominated volumetrically by squids and fishes.

Significant feeding differences between size classes were detected by the Tyler chi-square feeding heterogeneity index (Table 2). Several groups of crustaceans and fishes demonstrated significant differences in frequency between size classes of vermilion snapper. Decapods were important prey for larger fish but dominated the diet of fish between 50 and 100 mm SL. Small crustaceans, such as copepods, stomatopods and amphipods decreased in relative number and volume in the diet with increasing size of predator, although amphipods remained a frequent food item in all size classes examined. Barnacles and cumaceans varied in relative volume of prey for different size classes, but cumaceans increased in frequency in larger fish. Although squids and fishes were eaten by most size classes, they were volumetrically most important in

Table 1

Food items that occurred with a frequency (*F*) of at least one percent of stomachs with food, or that made up at least one percent of the total number (*N*) or volume (*V*) of food in vermilion snapper (*Rhomboplites aurorubens*) stomachs (*n*=255). All higher taxonomic groupings of prey are listed, regardless of their frequency, number, or volume. P=planktonic, H=holoplanktonic, D=demersal zooplankton; B=benthic, E=epifaunal, I=infaunal; N=nektonic.

Taxon and prey item	*F*	*N*	*V*
Cnidaria			
nematocysts	1.2	0.1	0.1
Annelida			
Total Polychaeta	11.9	1.8	2.5
Phyllodoce longipes (I)	3.2	0.4	0.3
Mollusca			
Total Gastropoda	5.1	0.9	0.3
Natica pusilla (B)	1.6	0.4	0.1
Total Pelecypoda	1.2	0.3	<0.1
Ervilia concentrica (I)	1.2	0.3	<0.1
Total Cephalopoda	5.9	0.4	43.8
Loliginidae undet.	4.4	0.3	22.4
Loligo plei (N)	0.8	<0.1	21.1
Crustacea			
Total Ostracoda	12.6	11.1	0.2
Ostracoda A (H)	11.1	11.0	0.2
Total Copepoda	43.5	13.4	0.5
Calanopia americana (D)	2.8	0.5	<0.1
Candacia curta (P)	2.8	0.4	<0.1
Centropages furcatus (H)	4.0	0.3	<0.1
Labidocera aestiva (P,B)	6.3	0.7	0.1
Labidocera sp. (H)	1.6	0.2	<0.1
Oncaea sp. (H)	1.6	0.3	<0.1
Sapphirina sp. (H)	1.2	0.1	<0.1
Temora stylifera (H)	9.5	1.6	<0.1
Temora turbinata (H)	21.7	5.3	0.2
Undinula vulgaris (H)	3.2	0.6	<0.1
Total Cirripedia	16.2	3.1	2.5
Barnacle larvae (D)	15.4	3.0	2.5
Total Stomatopoda	9.9	2.2	1.0
Stomatopod larvae (P)	8.3	2.0	0.8
Stomatopoda adults (D)	1.2	0.1	0.2
Total Mysidacea	16.6	5.2	0.8
Anchialina typica (D)	1.2	0.1	<0.1
Bowmaniella portoricensis	9.9	1.0	0.3
Bowmaniella sp.	1.2	0.1	<0.1
Mysidopsis bigelowi (B)	1.2	0.1	<0.1
Promysis atlantica (P)	2.0	3.6	0.4
Total Cumacea	14.6	7.4	1.1
Cyclaspis varians (D)	5.5	0.7	0.1
Oxyurostylis smithi (D)	11.1	5.9	0.8
Total Isopoda	2.0	0.2	<0.1
Total Amphipoda	47.4	13.7	1.8
Gammaridea			
Ampelisca abdita (D)	1.2	0.1	<0.1
Ampelisca vadorum (D)	2.0	0.1	0.1
Corophiidae undet.	1.6	0.3	<0.1
Gammaropsis sp.	2.0	0.2	<0.1
Lysianopsis alba	1.6	0.1	<0.1
Photis sp.	1.2	0.1	<0.1
Rudilemboides naglei	2.0	0.3	<0.1
Synchelidium americanum	1.6	0.1	<0.1
Tiron tropakis (I)	2.0	0.1	<0.1
Caprellidea			
Caprellidae undet.	1.6	0.1	<0.1
Phtisica marina (P,B)	14.6	2.4	0.3
Hyperiidea			
Euprone sp. (H)	1.2	0.1	<0.1
Hyperiidae undet. (H)	5.1	0.5	0.1
Hyperiidea undet. (H)	5.9	0.7	<0.1
Lestrigonus bengalensis (H)	17.8	4.1	0.2
Lycaea sp. (H)	1.2	0.1	<0.1
Phronima sp. (H)	2.0	0.7	0.3
Phronima sedentaria (H)	1.6	0.3	<0.1
Phronimella elongata (H)	2.0	0.8	<0.1
Simorhynchotus sp. (H)	1.6	0.2	<0.1
Total Decapoda	56.5	20.8	11.7
Decapoda undet. zoea (P)	3.2	0.2	<0.1
Decapoda undet. larvae	2.0	0.2	<0.1
Natantia undet. zoea (P)	2.4	0.3	<0.1
Natantia undet. shrimp	12.2	1.3	1.4
Penaeidea			
Lucifer faxoni (H)	14.2	8.5	0.8
Penaeidae zoea (P)	1.2	0.1	<0.1
Sicyonia typica	1.2	0.1	2.0
Solenocera atlantidis	1.2	0.1	0.2
Caridea			
Caridea undet. shrimp	1.2	0.1	<0.1
Leptochela sp.	3.2	0.3	0.2
Leptochela papulata	16.6	2.2	3.0
Ogyrides sp.	2.0	0.2	0.2
Processa sp.	1.2	0.1	0.1
Anomura			
Albunea parettii	4.7	1.4	0.2
Ranilia muricata zoea (P)	2.0	0.2	<0.1
Brachyura			
Brachyura undet. zoea (P)	4.0	0.4	<0.1
Brachyura undet. megalopae (P,B)	7.9	1.1	0.2
Brachyura undet. crab	5.5	0.4	0.4
Calappidae zoea (P)	2.0	0.2	0.1
Ovalipes sp. (P,B)	1.2	0.1	0.4
Pinnotheridae zoea (P)	2.4	0.3	<0.1
Portunidae megalopae (P,B)	4.4	0.6	0.1
Portunidae crab (P,B)	2.8	0.2	0.2
Portunus sp. (P,B)	2.0	0.2	0.2
Xanthidae crab (B)	1.6	0.1	0.3
Sipunculida (B)	1.2	0.1	<0.1
Chaetognatha (P)	13.8	1.9	0.3
Chordata			
Thaliacea			
Doliolida undet. (H)	8.3	7.4	1.2
Larvacea	0.8	<0.1	<0.1
Cephalochordata			
Branchiostoma caribbaeum (D)	3.2	0.4	0.1
Total Teleostei	27.7	6.9	32.2
Anguilliformes	0.4	<0.1	1.3
Prionotus sp. larvae (P)	1.2	4.2	2.6
Sardinella aurita (N)	1.6	0.2	12.4
Teleostei undet. eggs (P)	2.0	3.0	<0.1
Teleostei undet. larvae (P)	3.2	0.4	0.6
Teleostei undet.	14.2	1.3	14.7

Table 2

Percent frequency occurrence (*F*), percent number (*N*), and percent volume (*V*) of higher taxonomic groups of food in the diet of vermilion snapper (*Rhomboplites aurorubens*), by length interval. An asterisk (*) indicates significant differences in prey frequency between one length interval and the adjacent larger interval, based on Tyler's (1979) test.

	Length intervals (mm SL)											
	1–50			51–100			101–150			>150		
Prey	*F*	*N*	*V*	*F*	*N*	*V*	*F*	*N*	*V*	*F*	*N*	*V*
Cnidaria (nematocysts)	—	—	—	—	—	—	2.9	0.3	0.9	—	—	—
Annelida												
Polychaeta	7.1*	1.6	2.1	10.9	0.4	2.8	13.3	2.2	2.8	10.9	1.6	2.2
Mollusca												
Gastropoda	7.1	0.9	0.2	1.8	0.1	<0.1	5.7	1.6	1.0	6.0	1.4	0.1
Pelecypoda	—	—	—	—	—	—	2.9	1.2	0.2	—	—	—
Cephalopoda	—	—	—	1.8	0.1	1.0	4.8	0.6	24.2	13.4	1.0	51.4
Crustacea												
Ostracoda	28.6	3.7	1.5	24.4*	29.1	3.6	7.6	1.0	0.1	3.0	0.2	<0.1
Copepoda	82.1	33.9	12.0	60.0	15.3	4.0	41.0*	13.1	1.0	16.4	1.9	<0.1
Cirripedia	21.4	6.1	4.2	20.0	1.3	0.5	11.4	2.6	2.5	18.0	4.8	2.6
Stomatopoda	25.0	2.8	9.6	20.0*	4.6	8.4	3.8	0.4	1.5	4.5	0.3	0.3
Mysidacea	21.4	1.9	4.6	12.3	0.7	0.5	20.0	16.0	4.0	11.9	1.5	0.1
Cumacea	3.6	0.9	0.8	7.3*	0.4	0.4	21.0	21.7	5.0	14.9	4.3	0.3
Isopoda	3.6	0.2	0.2	—	—	—	—*	—	—	6.0	0.5	0.1
Amphipoda	35.7	20.3	12.7	49.1	12.9	6.2	49.5	14.2	4.0	46.3	11.5	0.9
Decapoda	35.7*	25.0	31.1	70.9*	29.4	42.0	52.4	14.1	19.0	58.2	14.3	7.9
Sipunculida	—	—	—	—	—	—	1.9	0.2	0.1	1.5	0.1	<0.1
Chaetognatha	21.4	1.6	3.4	10.9	1.3	0.9	11.4	2.3	0.8	16.4	2.3	0.1
Chordata												
Thaliacea	3.6	0.2	0.4	9.1	1.5	1.6	5.7	2.0	0.4	13.4	24.9	1.3
Larvacea	—	—	—	—	—	—	—	—	—	3.0	0.2	<0.1
Cephalochordata	—	—	—	1.8	0.1	0.2	2.9	1.0	0.2	6.0	0.4	0.1
Teleostei	7.1*	0.7	17.1	43.6*	2.9	27.8	21.9*	5.5	32.4	38.8	28.8	32.7
Examined stomachs with food:		28			55			105			67	
Mean SL (mm) of fish with food:		36.4			77.8			131.1			168.1	

the diet of larger vermilion snapper (Table 2). Squids and fishes were 76% of the prey volume for all vermilion snapper, but 84.1% of the prey volume (and only 29.8% by number) of fish greater than 150 mm SL.

Vermilion snapper fed sparingly on invertebrates closely associated with the reef habitat and collected in suction and grab samples during the day (Table 3). Electivity values were negative (usually –1.00) for all dominant species in benthic samples. Polychaete species that dominated those samples consisted mainly of tube-reef building species (*Filograna implexa*) and species associated with sponges and corals (*Exogone dispar* and *Syllis spongicola*) (Gardiner, 1975; Wendt et al., 1985); none of these were consumed by vermilion snapper. On the other hand, many species that dominated numerically in the diet (e.g., *Oxyurostylis smithi* and *Lucifer faxoni*) were collected in benthic samples but were not a major component of the daytime reef fauna. Because abundance of these species in benthic samples was so low, electivity values were positive. Those species that were higher in relative abundance in stomachs than in benthic samples (e.g., *O. smithi*) may have been consumed in the water column during periodic emergence.

As in the case of benthic samples, most species of cumaceans, mysids, stomatopods, and decapods that dominated samples from the sled were not as relatively abundant in stomach samples (Table 4). Most electivity values were negative; however, fewer absences from stomach samples (E=–1.00) occurred with the dominant species from sled samples than with the benthic samples. The mysid *Promysis atlantica* at the inner shelf (E=0.77) and the decapod *Lucifer faxoni* at the middle shelf (E=0.63) were dominant species in sled samples that were positively elected as prey. *Lucifer faxoni* was by far the most abundant species in

Table 3

Relative abundance (percent of total number of individuals, *N*) and electivity values (*E*) for dominant benthic species in suction and grab samples and in vermilion snapper (*Rhomboplites aurorubens*) stomachs. Dominant species for each type of sample (benthic or stomach) include those that ranked in the five most abundant species in any depth zone for that type of sample.

	Inner shelf			Middle shelf			Outer shelf		
	Relative abundance			Relative abundance			Relative abundance		
	Fish stomachs	Benthic samples	*E*	Fish stomachs	Benthic samples	*E*	Fish stomachs	Benthic samples	*E*
Dominant species — benthic samples									
Chone americana	—	0.33	–1.00	—	0.81	–1.00	—	0.59	–1.00
Erichthonius brasiliensis	0.09	2.89	–0.94	—	0.30	–1.00	—	0.13	–1.00
Erichthonius sp. A	—	0.08	–1.00	—	—	—	—	3.75	–1.00
Exogone dispar	0.44	3.71	–0.79	—	0.47	–1.00	—	0.01	–1.00
Filograna implexa	—	20.42	–1.00	—	63.87	–1.00	—	21.90	–1.00
Luconacia incerta	—	3.27	–1.00	0.05	1.03	–0.90	—	0.18	–1.00
Malacoceros glutaeus	—	0.41	–1.00	—	0.81	–1.00	—	0.02	–1.00
Phyllochaetopterus socialis	—	0.21	–1.00	—	0.12	–1.00	—	12.40	–1.00
Pista palmata	—	0.09	–1.00	—	0.08	–1.00	—	8.60	–1.00
Podocerus sp. A	—	2.87	–1.00	—	0.27	–1.00	—	0.14	–1.00
Spiophanes bombyx	—	0.39	–1.00	—	0.46	–1.00	—	5.81	–1.00
Syllis spongicola	—	2.14	–1.00	—	1.90	–1.00	—	1.38	–1.00
Total	0.53	36.81		0.05	70.12		0.00	54.92	
Dominant species — stomachs									
Bowmaniella portoricensis	0.18	0.09	0.32	1.80	0.10	0.89	0.50	0.22	0.37
Leptochela papulata	0.35	0.04	0.81	3.45	0.09	0.95	1.86	0.12	0.88
Lucifer faxoni	12.05	0.18	0.97	9.79	0.01	0.99	0.62	0.04	0.87
Oxyurostylis smithi	15.74	0.67	0.92	2.46	0.14	0.89	—	—	—
Phtisica marina	0.09	0.01	0.75	1.80	0.03	0.97	7.20	0.40	0.90
Phyllodoce longipes	1.14	0.06	0.89	0.05	0.04	0.10	0.12	0.20	–0.22
Promysis atlantica	11.79	—	1.00	0.05	<0.01	0.88	—	—	—
Rhudilemboides naglei	1.06	0.05	0.91	—	<0.01	–1.00	—	0.04	–1.00
Total	42.40	1.10		19.40	0.42		10.30	1.02	
Number of stomachs with food:		49			138			68	

sled samples at the inner shelf and was also abundant in stomach samples but demonstrated negative electivity at the inner (E=–0.53) and outer (E=–0.39) shelf sites. At the middle shelf, *L. faxoni* was the most abundant species in the analyzed taxa in fish stomachs and ranked third in abundance in sled samples. Many of the species that were high in relative abundance in fish stomachs also occurred in sled samples; however, electivity values were not always positive. The relative abundance of cumaceans, mysids, stomatopods, and decapods in fish stomachs, compared with their abundance in sled samples, indicated that vermilion snapper often selected crustaceans in higher proportions than was their availability to the plankton sled at night (Table 4). Included were several orders of Crustacea, particularly the cumacean *Oxyurostylis smithi* and the mysid *Promysis atlantica* at inner shelf stations, the decapods *Lucifer faxoni* and *Leptochela papulata* at middle shelf stations, and the decapods *Leptochela papulata* and *Solenocera atlantidis* at the outer shelf.

Comparing dominant species in the diet of vermilion snapper with their relative abundance in benthic (lower half of Table 3) and sled (lower half of Table 4) samples indicated high positive selectivity for most prey species from both environments sampled. For suction and grab samples, this was due to the extremely low abundance (<1%) of all the dominant prey in the benthos. For the sled samples, many dominant prey species were also dominant in the environment.

Discussion

The vermilion snapper is well adapted to foraging in the water column (Davis and Birdsong, 1973; Grimes, 1979). Grimes (1979) reported that the diet of vermilion snapper was dominated by planktonic organisms

Table 4

Relative abundance (percent of total number of individuals, *N*) and electivity values (*E*) for dominant species of decapods, cumaceans, mysids and stomatopods in sled samples and vermilion snapper (*Rhomboplites aurorubens*) stomachs. Dominant species for each type of sample (sled or stomach) include those that ranked in the five most abundant species of decapods, cumaceans, mysids and stomatopods in any depth zone for that type of sample.

	Inner shelf			Middle shelf			Outer shelf		
	Relative abundance			Relative abundance			Relative abundance		
	Fish stomachs	Sled samples	*E*	Fish stomachs	Sled samples	*E*	Fish stomachs	Sled samples	*E*
Dominant species — sled samples									
Bowmaniella portoricensis	0.30	5.56	−0.90	5.52	10.45	−0.31	4.30	5.45	−0.12
Lucifer faxoni	20.57	66.77	−0.53	29.93	6.79	0.63	5.38	12.29	−0.39
Mysidopsis furca	—	0.67	−1.00	0.17	4.49	−0.93	—	22.28	−1.00
Neopontonides beaufortensis	—	4.10	−1.00	—	3.35	−1.00	—	0.77	−1.00
Periclimenes iridescens	—	3.18	−1.00	—	3.75	−1.00	—	0.84	−1.00
Pontophilus gorei	—	—	—	—	0.03	−1.00	—	7.33	−1.00
Promysis atlantica	20.12	2.57	0.77	0.17	7.24	−0.95	—	0.63	−1.00
Thor manningi	—	0.05	−1.00	—	0.43	−1.00	—	2.44	−1.00
Total	40.99	82.90		35.15	36.63		9.68	52.03	
Dominant species — stomachs									
Bowmaniella portoricensis	0.30	5.56	−0.90	5.52	10.45	−0.31	4.30	5.45	−0.12
Cyclaspis varians	1.05	0.05	0.91	3.18	1.45	0.37	—	0.41	−1.00
Lucifer faxoni	20.57	66.77	−0.53	29.93	6.79	0.63	5.38	12.29	−0.39
Leptochela papulata	0.60	0.39	0.21	10.54	2.07	0.67	16.13	1.61	0.82
Oxyurostylis smithi	26.88	1.38	0.90	7.52	1.19	0.73	—	0.42	−1.00
Promysis atlantica	20.12	2.57	0.77	0.17	7.24	−0.95	—	0.63	−1.00
Solenocera atlantidis	—	—	—	—	0.03	−1.00	4.30	1.12	0.59
Total	69.52	80.82		56.86	32.56		30.11	22.70	
Number of stomachs with food:		49			138			68	

and he noted that the diet of juveniles (<100 mm TL) was dominated volumetrically by copepods. In the present study, decapods dominated the diet volume of fish less than 101 mm SL. The feeding habits of vermilion snapper changed considerably with size in the present study, although pelagic prey dominated in all size classes. As vermilion snapper grows, it switches from a diet of many small crustaceans, to a diet dominated by a few large cephalopods, fishes, or decapod crustaceans. The switch to different prey taxa and to fewer, larger prey individuals was similar to ontogenetic diet changes noted by Sedberry (1983) for several demersal fishes from the outer continental shelf. In contrast, Schmitt and Holbrook (1984) found that black surfperch (*Embiotoca jacksoni*) switched to larger prey, but that gross taxonomic composition of the diet did not change and that surfperch continued to feed on macrocrustaceans, in spite of growth and changes in body size and foraging behavior. Vermilion snapper, like many other fishes (Sedberry, 1983), apparently becomes capable of taking larger prey as it grows and switches from picking plankton to pursuing and capturing active nektonic species, such as Spanish sardine (*Sardinella aurita*), and squids.

Grimes (1979) suggested that vermilion snapper is a nocturnal forager and that selective feeding by vermilion snapper on demersal zooplankton, such as cumaceans, indicates nocturnal foraging. These crustaceans are infaunal in the sand or epifaunal in reef crevices during the day but emerge at night (Anger and Valentin, 1976; Alldredge and King, 1985), becoming prey for vermilion snapper and other fishes that forage on near-bottom zooplankton at night. Demersal zooplankton demonstrating nocturnal emergence and found in the diet of vermilion snapper included syllid polychaetes, some calanoid copepods, cumaceans, amphipods, decapods, barnacle and stomatopod larvae, chaetognaths and cephalochordates (Williams and Bynum, 1972; Fincham, 1974; Anger and Valentin, 1976; Hobson and Chess, 1976; Alldredge and King, 1977; Hammer, 1981; Alldredge and King, 1985; Cahoon and Tronzo, 1988). These taxa are dominant members of the demersal zooplankton (Porter and Porter, 1977) and emerge from benthic habitats at night when they

feed, molt, reproduce or disperse (Alldredge and King, 1985). This behavior makes them subject to intense predation by vermilion snapper or other specialized nocturnal predators (Robertson and Howard, 1978).

Demersal zooplankton are approximately as abundant as holozooplankton on the continental shelf off North Carolina (Cahoon and Tronzo, 1992), and the nocturnal emergence of demersal zooplankton probably increases overall food availability at night, while allowing planktivorous fishes to feed under the cover of darkness. Small vermilion snapper are prey for diurnal lutjanids and crepuscular serranids (South Carolina Wildlife and Marine Resources Department[1]; Parrish, 1987; Sedberry, 1988) and would be subject to predation during the day.

Vermilion snapper also consumes holoplanktonic species such as copepods, hyperiids, *Lucifer faxoni*, and doliolids, as well as nektonic squids and fishes. As noted by Grimes (1979), squids were especially important in the diet of large juveniles and adults. These larger size classes of vermilion snapper also fed more on fishes, which included schooling pelagic species such as Spanish sardine, *Sardinella aurita*. Spanish sardine makes up 9.4% of fishes caught in trawls on the southeastern continental shelf[2]. Predation by vermilion snapper on nektonic foragers such as squids and Spanish sardine provides a trophic link between the pelagic nekton and the reef. In the role as an abundant consumer of nekton, holoplankton, and demersal zooplankton, vermilion snapper may be important in transferring energy from benthic sand habitats and the water column to the reef, in the form of feces. Feces that disintegrate just above the reef provide fine particles and nutrients for filter- and suspension-feeders, and fecal pellets that are less refractile can be used directly by small crustaceans and other organisms living in the reef (Rothans and Miller, 1991). Vermilion snapper appear to be relatively inactive during the day, hovering or moving slowly in large schools along the reefs within a meter of the bottom (pers. observ. by GRS from *Sea Link I*). By feeding in the water column at night and swimming just above the reef during the day, vermilion snapper enhances the transfer of this organic matter to the benthos. During these diurnal resting periods, vermilion snapper probably deposit feces, derived from water column nocturnal foraging, onto the reef. Bray et al. (1981) also found that a planktivorous reef fish functioned as a trophic link between the plankton and benthos by importing organic carbon to the reef in the form of feces. Meyer and Schultz (1985) found that grunts (*Haemulon* spp.) feeding on sand flats transferred significant amounts of nutrient and organic matter to reefs, thus enhancing coral growth.

Benthic polychaetes ranking high in relative abundance in the suction and grab samples were not important in the diet of vermilion snapper, and electivity values for all dominant species in the benthic samples were negative. The dominant crustaceans in benthic samples were epibenthic species such as the corophoid amphipod, *Erichthonius brasiliensis*, and the caprellid amphipod *Luconacia incerta* which attach to sessile invertebrates and are usually closely associated with the reef substratum (McCain, 1968; Bousfield, 1973). Because *E. brasiliensis* and caprellid amphipods have also been found in nocturnal zooplankton samples (Williams and Bynum, 1972; Hobson and Chess, 1976), they may be consumed by predators at night in the water column. Some motile benthic crustaceans such as mysids, cumaceans, and decapods that were common in vermilion snapper stomachs were much higher in relative abundance in stomachs than in benthic samples. Vermilion snapper apparently prey on these benthic crustaceans during their periodic migrations into the water column. The polychaetes that dominated benthic samples apparently do not undertake such migrations.

Vermilion snapper feed on many of the same prey species as the benthic-feeding sparid *Stenotomus chrysops*, an abundant demersal fish of the continental shelf (Sedberry and Van Dolah, 1984; Sedberry, 1988; Sedberry et al.[2]). Submersible observations indicate that *S. chrysops* feeds during the day on the benthos, living in sand adjacent to reef habitat, whereas vermilion snapper were not observed to forage in this manner. Vermilion snapper apparently consumes sand-dwelling benthos at night, when they emerge from the bottom, while *S. chrysops* feeds on benthos during the day when demersal zooplankton has burrowed in the bottom. This provides a temporal partitioning of prey resources between these two dominant species of reef-associated fish.

For reef fishes of the southeastern shelf, there is large variation in the degree of dependence on hard substrate as a habitat for prey (Sedberry, 1985, 1987, 1988). Of the three most abundant species in our reef trawl catches (*S. chrysops*, *Haemulon aurolineatum*, and *R. aurorubens*), all occur over sand bottom, although they are much more abundant over reefs (Wenner, 1983; Sedberry and Van Dolah, 1984). The

[1]South Carolina Wildlife and Marine Resources Department. 1984. Final Report. South Atlantic OCS area living marine resources study. Phase III. Volume 1. Prepared by Marine Resources Research Institute, SCWMRD, for Minerals Management Service, Washington, D.C., under contract No 14-12-0001-29185, 223 p.

[2]Sedberry, G. R., C. A. Barans, C. A. Wenner, and V. G. Burrell Jr. The ichthyofauna of sandy bottom habitat on the continental shelf off the southeastern U.S. Manuscr. in prep.

former two species feed primarily on sand bottom benthos (Sedberry, 1985, 1988). Although *H. aurolineatum* and *S. chrysops* feed heavily on sand infauna and are not completely dependent on reef habitat, vermilion snapper is more restricted to reef habitat and does not range far from a home reef (Fable, 1980). Wenner (1983) collected only 2 specimens in 11 trawling tows in sand bottom habitat on the southeastern continental shelf, whereas *S. chrysops* and *H. aurolineatum* are dominant species in sand habitats (Wenner, 1983; Sedberry[2]). Vermilion snapper did not, however, feed directly on reef fauna, and its attraction to reefs may be behavioral rather than trophic.

Although vermilion snapper feeds extensively on demersal zooplankton, many individuals also feed opportunistically on concentrations of holoplankton that occur on the southeastern shelf. Copepods, such as *Undinula vulgaris* and especially *Temora turbinata*, that were frequent food items for vermilion snapper are epipelagic oceanic species that are transported landward across the continental shelf (Hopkins et al., 1981). Off the southeastern United States, upwelling at the shelf edge and cross-shelf transport of deep, highly productive oceanic water provide mechanisms for shoreward movement of shelf-edge biota, including copepods (*U. vulgaris*, *T. turbinata*, *Oncaea* spp.), doliolids, sergestid decapods (e.g., *Lucifer faxoni*), and other abundant oceanic zooplankters (Yoder et al., 1983; Paffenhöfer et al., 1984). Vermilion snapper, one of the most abundant fishes at shelf edge depths (Sedberry and Van Dolah, 1984), apparently takes advantage of this abundant resource and performs the function of transferring some of this oceanic productivity to bottom habitats on the continental shelf.

In conclusion, vermilion snapper feeds on a variety of prey above the substrate, much of which is demersal zooplankton. Although it is unknown how much of the biomass of the daytime benthos is composed of nocturnally emerging demersal zooplankton, it is apparent that these organisms are an important food source for vermilion snapper and other reef fishes. Demersal zooplankton, such as mysids, cumaceans, ampeliscid amphipods, and certain decapods, composed at least eight percent of the total volume of food for vermilion snapper in the present study and are more important in the diet of smaller size classes. While a small contribution, these benthic species provide a feeding opportunity for vermilion snapper and may be more important in the diet during periods of low productivity of holoplankton. Nektonic fishes and cephalopods provide the greatest volume of food for larger vermilion snapper. Because it is prey for other reef predators, vermilion snapper is an important trophic link among several habitats on the southeastern continental shelf.

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Latitudinal variation in the birth timing of captive California sea lions and other captive North Pacific pinnipeds

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Abstract.—The relationship between latitude and birth timing was assessed for captive-born California sea lions (*Zalophus californianus*), northern (Steller) sea lions (*Eumetopias jubatus*), northern fur seals (*Callorhinus ursinus*), and Pacific harbor seals (*Phoca vitulina richardsi*) from zoos and aquaria in the United States, Canada, South Korea, and New Zealand. The births of 466 viable California sea lion pups demonstrated highly significant, negative and curvilinear latitudinal variation in birth timing. Over the latitudinal range of captive births, this variation accounted for a change of approximately –0.6 days/°latitude. Furthermore, the variances of the mean dates of birth for the largest 18 captive populations were significantly dependent upon latitude; shorter birthing periods occurred at higher latitudes. Northern sea lions (n=9) had a similar, but non-significant latitudinal relationship in which birthing dates occurred approximately 30 days later. No significant relationship between latitude and birthing date was found for northern fur seals (n=13). The birth dates of 110 viable Pacific harbor seal pups had highly significant, positive and curvilinear latitudinal variation, similar to that previously described for this subspecies in the wild between 30° and 47°N. Pupping dates for each species in captivity were comparable to those found for wild populations of North Pacific pinnipeds. The described latitudinal variation and the temporal consistency between captive and wild populations of California sea lions and Pacific harbor seals support the hypothesis that photoperiod response maintains specific birth timing in these species.

Captive pinnipeds are not only of educational and entertainment value to zoo and aquarium visitors, but also offer an exceptional opportunity to examine temporal aspects of their reproductive biology. Zoo records usually document birth dates, outcomes, and parental histories which are difficult, if not impossible, to obtain in field studies. Moreover, captive animals live in environments where availability of food is relatively constant and any movements (i.e., transfers between facilities) are well documented.

A recent census (Asper et al., 1988) identified 924 pinnipeds in captivity in North America. Of these, four North Pacific species—the California sea lion (*Zalophus californianus*), the northern or Steller sea lion (*Eumetopias jubatus*), the northern fur seal (*Callorhinus ursinus*), and the harbor seal (*Phoca vitulina*)—represented 91 percent (n=837). These species breed successfully in captivity over wide latitudinal ranges.

Standard reviews of the pinnipedia (Mate, 1979; Mate and Gentry, 1979; Odell, 1981; Schusterman, 1981; King, 1983) identify fixed seasons of birth for each of the North Pacific sea lions. Moreover, Bigg (1973) found that captive California sea lions at the London Zoo (51°N) breed at the same time as the parent population in California (33°N). In contrast, Schusterman et al. (1982) noted that births of California sea lion pups at Sea Life Park, Hawaii (21°N), occurred significantly later than births in California at Marineland (34°N) and Marine World (38°N).

Regional or latitudinal variation in birth timing, or both, has been reported for the harbor seal (Bigg, 1969a; Temte et al., 1991) and the grey seal (*Halichoerus grypus*: Coulson, 1981). Likewise, Temte (1985) demonstrated a 14-day shift in the mean date of pupping for northern fur seals between colonies on St. George Island, Alaska (57°N), and San Miguel Island, California (34°N). These variations in birth timing have been interpreted as 1) selection acting on discrete populations to match reproductive efforts with seasonal constraints (Bigg, 1973), 2) responses to latitudinally changing temporal cues, such as temperature or photoperiod (Coulson, 1981; Temte, 1985), or 3) a combination of the above (Temte et al., 1991; Boyd, 1991).

This study identifies and defines the extent of latitudinal variation in birth timing of captive California sea lions, northern sea lions, northern fur seals and Pacific harbor seals from birthing records available from zoos and aquaria scattered across a wide latitudinal range. Comparisons of birthing periods were made, where applicable, between captive and wild populations.

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Methods

The data

Data for this analysis were initially compiled from the Marine Mammal Inventory Report (MMIR), a registry of captive marine mammals maintained by the Office of Protected Resources, National Marine Fisheries Service. To comply with the Marine Mammal Protection Act, U.S. facilities that exhibit marine mammals submit yearly summaries of their captive populations, including animal identification, sex, age, date of acquisition or birth, origin, and current status. The birth dates of 527 California sea lions born in captivity at 41 locations, 9 northern sea lions born at two locations, 20 northern fur seals born at three locations, and 125 Pacific harbor seals born at 13 locations were extracted from this registry (Fig. 1). Seventy-one percent of birth date and outcome (viable pup, non-viable pup, stillborn pup) data were independently verified by contacting the appropriate facility. Of the data verified, 98.6% were accurately reported on the MMIR (Temte, 1993).

Eighty-three additional captive births of California sea lions, not reported on the MMIR, were provided by the facilities. Seven additional birth dates of captive northern fur seals were obtained from the report of Bigg (1984). Data on two births of harbor seals were obtained from the Vancouver Aquarium (Vancouver, British Columbia). To assess the effect of a major latitudinal translocation, birthing dates of 11 California sea lions born at the Auckland Zoo, New Zealand (37°S) were obtained. All cows at this facility could be traced, from zoo records, to the California Channel Islands population.

Pup identification, location, latitude, date of birth, sex, and birth outcome were entered into the database. Dates of birth were converted into numerical equivalents by using a sequential astronomical calendar (day 0 = December 21; see Temte, 1985). Birth outcomes were rated as stillbirth (pup born dead), non-viable (pup lived less than one day), or viable (pup lived at least one day).

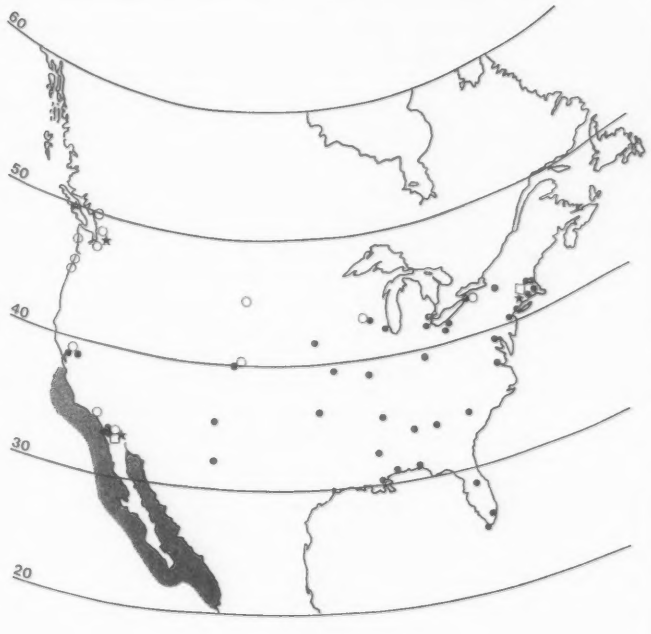

Figure 1

Locations of zoos, aquaria and marine zoological parks where captive births of California sea lions (filled circles), northern sea lions (open boxes), northern fur seals (stars) and Pacific harbor seals (open circles) have been reported. Sea Life Park, Waimanalo, Hawaii (21°20'N: CSL), Auckland Zoo, Auckland, New Zealand (37°52'S: CSL) and Seoul Grand Park, Seoul, South Korea (37°31'N: PHS) are not shown. The shaded area indicates the natural breeding range of the California sea lion.

Statistical analysis

Data were treated separately for each species. Comparisons were made between the mean dates of birth for stillborn, non-viable, and viable pups by using the appropriate parametric or non-parametric statistic.

The birth dates of viable pups were assessed for latitudinal variation. Least squares linear regression models were fitted to the latitude-date data, and where necessary, orthogonal polynomials were used to meet statistical assumptions. Because the intra-colony variance of the mean date of birth for California sea lions was related to latitude, regressions were performed with and without weighting to correct for unequal variances. Orthogonal polynomial regression models using (latitude minus mean latitude)^2 as the quadratic term were chosen to reduce correlation between the linear and quadratic terms and to better estimate the linear coefficient (Snedecor and Cochran, 1980). To assess differences between California and northern sea lions, analysis of covariance (ANCOVA: Kleinbaum and Kupper,

1978) was utilized to control for differences in latitudinal distributions.

Results

California sea lion

The captive births of 610 California sea lions were evaluated (Table 1). Stillborn and non-viable pups accounted for 122 births (20.0%). Stillbirths occurred as early as 1 January and as late as 1 August. Whereas no significant difference was found between the timing of stillbirths and non-viable births (Kruskal-Wallace: df=1; H=0.371; NS), stillborn and non-viable pups were born an average of 28.0 days earlier than viable pups, this difference being highly significant (Kruskal-Wallace: df=1; H=70.49; P<0.001). Hence, only viable births (n=466; 22 pups with estimated birth dates were excluded) were examined for latitudinal variation.

Viable births occurred over a 21°46' latitudinal range, between 21°20'N and 43°06'N. These were normally distributed over the birthing period (Fig. 2), occurring as early as 30 April and as late as 1 September. Mean dates of pupping for individual colonies occurred from 27 May to 25 June. The mean date of birth for all viable pups was 12 June ± 13.5 days (± SD).

The variances of the mean birth dates were calculated for the 18 largest captive colonies (each with at least 6 viable births). A significant latitudinal gradient existed for this variance (Fig. 3), with a decrease of 5.6 days^2 for each degree of northward displacement.

A plot of pupping date versus latitude indicated that latitude variation occurred within captive California sea lions (Fig. 4). A second order orthogonal polynomial model (Table 2) was highly significant (r^2=0.206; $F_{[2,463]}$=60.23; P<0.0001) and estimated a negative latitudinal slope of approximately –0.6 days/°latitude over

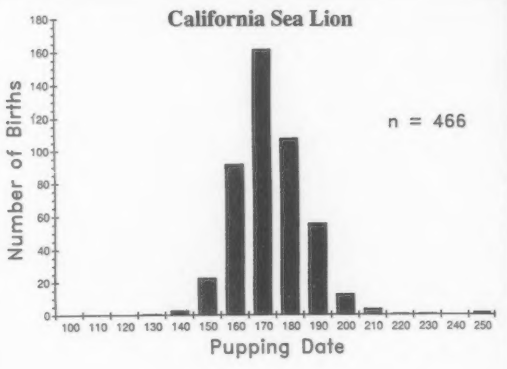

Figure 2

Frequency of viable California sea lion births at all facilities during 10-day periods from 31 March (day 100) to 6 September (day day 259). Dates are numbered sequentially from 21 December.

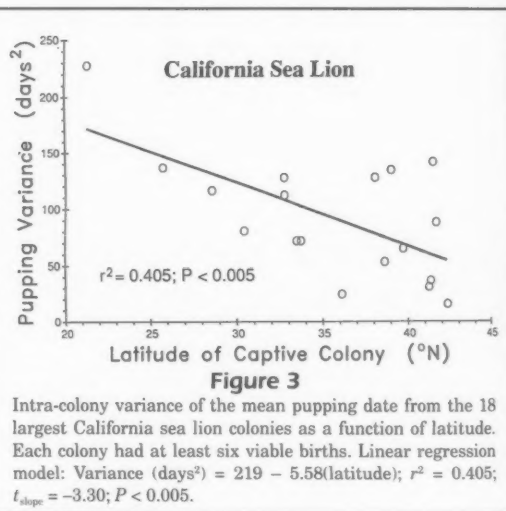

Figure 3

Intra-colony variance of the mean pupping date from the 18 largest California sea lion colonies as a function of latitude. Each colony had at least six viable births. Linear regression model: Variance (days^2) = 219 – 5.58(latitude); r^2 = 0.405; t_{slope} = –3.30; P < 0.005.

Table 1

Numbers, mean dates (month/day; sequential day number), and standard deviations of viable births, non-viable births, and stillbirths in captive North Pacific pinnipeds.

Species	Type	n	Percent	Mean date	SD (days)
California sea lion	Viable	488	80.0	6/12 (172.7)	13.5
	Non-viable	50	8.2	5/16 (145.6)	29.4
	Stillborn	72	11.8	5/14 (144.0)	46.1
Northern sea lion	Viable	9	100.0	7/09 (199.8)	16.5
Northern fur seal	Viable	13	48.1	7/10 (200.6)	14.8
	Stillborn	14	51.9	6/23 (184.2)	60.0
Pacific harbor seal	Viable	110	88.0	5/20 (150.3)	36.7
	Non-viable	5	4.0	5/11 (140.8)	47.5
	Stillborn	10	8.0	5/11 (140.8)	34.0

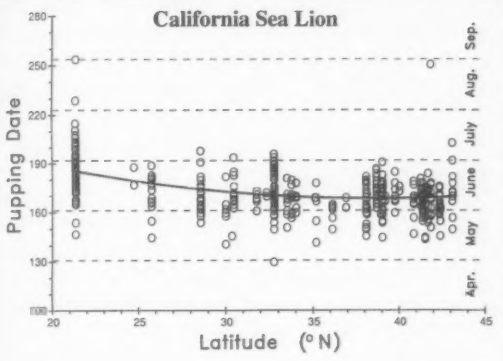

Figure 4
The birth timing of viable California sea lions as a function of latitude. See Table 2 for regression model.

the range of data. Weighting of the regression to correct for the unequal variances did not appreciably change the parameter estimates.

Eleven pups were born at the Auckland Zoo (Auckland, New Zealand: 37°S) from 28 November to 23 December and had a mean birth date of 11 December. The polynomial model predicted that birthing at 37°N should occur on 6 June. Therefore, California sea lions translocated to the Southern Hemisphere experienced an approximate 6-month shift in birth timing.

Climatic conditions did not appear to significantly affect the timing of birth. For example, colonies at similar latitudes (Vallejo, California: 37°47'N and Kansas City, Missouri: 39°07'N) maintained similar pupping schedules despite having vastly different seasonal temperature cycles. Following an adjustment for latitude, no significant difference was found between the birth timing of male and female pups.

Northern sea lion

The captive births of nine northern sea lions occurred at 32°45'N and 41°21'N (latitudinal range=8°36'). All births were of viable pups. The mean date of birth was 9 July ± 16.5 days (± SD; Table 1). Although the plot of pupping date vs. latitude was similar to that for the California sea lion, a simple linear regression model (Table 2) failed to demonstrate significant latitudinal effects in this species (r^2=0.271; $F_{[1,7]}$=2.60; P=0.23); the lack of significance was possibly due to the small sample size. However, when northern sea lion data were combined with those of the California sea lion in a multiple linear regression model, no significant differences were found between the latitudinal slopes of the two species. Analysis of covariance identified significantly later birthing (P<0.001) for northern sea lions, occurring 30 days after California sea lions.

Northern fur seal

The captive births of 27 northern fur seals were reported from four locations between 32°45'N and 49°07'N (latitudinal range=16°22'). Slightly more than half of the pups were stillborn (Table 1). The mean date of birth of viable pups was 10 July ± 14.8 days (± SD). No significant latitudinal variation was detected in birth timing (Table 2: r^2=0.174; $F_{[1,11]}$=2.32; NS).

Pacific harbor seal

The birth dates of 127 captive-born Pacific harbor seals were identified from 14 locations between 32°45'N and 49°18'N (latitudinal range=16°33'). The birth outcomes of two pups were not known and they were excluded from further analysis. Of the remaining 125 births, 88.0% were of viable pups (Table 1). The mean date of birth for viable pups was 20 May ± 36.7 days (± SD). No significant differences in the annual birth timing

Table 2
Regression models for latitudinal variation in birth timing of North Pacific pinnipeds.

Species	n	Mean latitude (° North)	*Linear regression estimate of $PD = a + b_1(L) + b_2(L - \bar{L})^2$ a	b_1 (CI) ±95%		b_2 (CI) ±95%		r^2	Significance
California sea lion	466	33.21	190	–0.61	(0.17)	0.057	(0.024)	0.206	0.0001
Northern sea lion	9	35.62	271	–2.00	(2.43)	—	—	0.271	NS
Northern fur seal	13	45.03	145	1.23	(1.59)	—	—	0.174	NS
Pacific harbor seal	110	41.60	–113	5.85	(1.10)	0.681	(0.065)	0.508	0.0001

*PD = Pupping date (sequential from 21 December); L = Latitude.

were found among viable, non-viable and stillborn pups.

A simple linear regression model defined significant latitudinal variation in birth timing of viable pups with a gradient of 4.10 days/°latitude; this slope was not significantly different from that reported by Temte et al. (1991) for colonies of wild Pacific harbor seals between 30° and 47°N. A second order polynomial model (Fig. 5; Table 2), however, was more statistically appropriate based on residual analysis. This model was highly significant (r^2=0.508; $F_{[2,107]}$=55.23; P<0.0001), defining a positive relationship between latitude and birth date.

Data regarding sex of pup (n=102), maternal age (n=88), and the previous annual cycle of the mother (pregnant vs. non-pregnant: n=89) were available for subsets of the harbor seals. When entered, either separately or as a group, into a multiple regression model adjusting for latitude, none of these parameters was found to significantly alter birth timing.

Discussion

Use of captive birth data

Events on rookery sites or pupping beaches hamper the estimation of true pupping seasons in pinnipeds. For example, premature pupping in the California sea lion occurs with increasing frequency over a 5-month period, melting into the normal pupping season between mid-May and the end of June (Delong et al., 1973). In addition, seasonal movements of animals from breeding to feeding sites may occur (Braham, 1974; Mate, 1975), potentially obscuring latitudinal variation of birthing if seasonal entrainment occurs at latitudes other than that of the rookery site. The use of captive populations permits accurate measurement of birth dates and birth outcomes, while controlling for potential latitudinal displacement during the reproductive year.

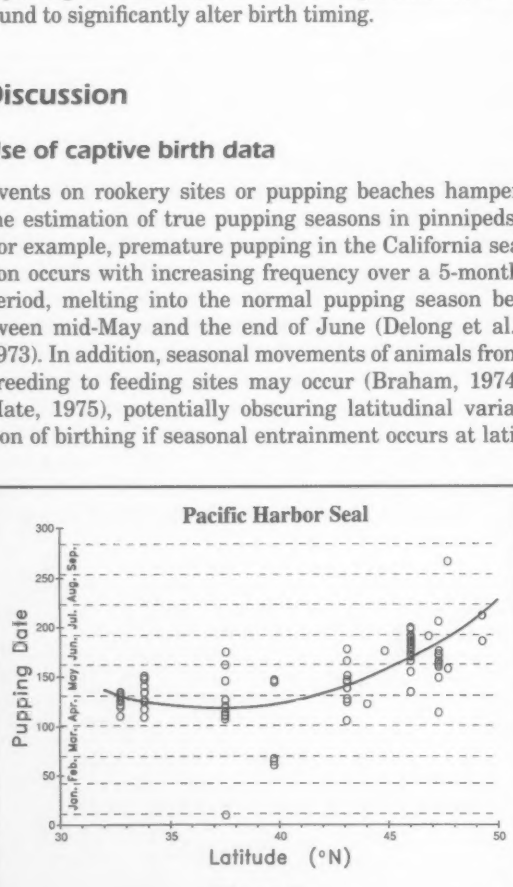

Figure 5
The birth timing of viable Pacific harbor seals as a function of latitude. See Table 2 for regression model.

California sea lion

This study confirms the report of Schusterman et al. (1982) and demonstrates that marked latitudinal variation occurs not only in the timing of birth, but also in the variance of the mean birth date. Furthermore, interpretations of results based on this data set representing 466 viable births from 41 locations, as compared to the three locations used by Schusterman et al. (1982), are far less sensitive to possible confounding effects induced by captivity or differences in climatic conditions. The extreme example of sea lions translocated to the Southern Hemisphere provides further evidence of strong latitudinal effect.

The birth timing of captives mirrors that of wild populations. Although a review by Mate (1979) concluded that pups, regardless of latitude, are born from mid-May to late-June over the range of California sea lion rookeries, slight latitudinal variation may exist. In Baja California, pupping occurs in late-June (Brownell et al., 1974), and Le Boeuf et al. (1983) reported a maximum pup count on 10 July at Los Islotes (28°N). Further to the north, at San Nicolas Island (33°15'N), pupping peaks during the first half of June (Peterson and Bartholomew, 1967; Odell, 1975; Heath and Francis[1,2]). Data from more northerly colonies are anecdotal in nature. For example, Braham (1974) reported a 2-week-old pup on 11 June at San Luis Obispo County, California (35°30'N).

As in captive sea lions, premature pupping in wild colonies occurred as early as January on San Nicolas and San Miguel Islands (Odell, 1970; Delong et al., 1973). Premature pups are probably represented in captivity by the stillborn and non-viable groups. The 20% rate of stillborn and non-viable pupping for captive sea lions is noteworthy considering the 5–16% rate of premature pupping reported for San Nicolas Island during years with high incidences of prematurity (Odell, 1970). Whereas pesticide exposure and disease

[1]Heath, C., and J. Francis. 1983. California sea lion population dynamics and feeding ecology with applications for management. Results of 1981–1982 research on Santa Barbara and San Nicholas Islands. U.S. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Southwest Fish. Sci. Center, P.O. Box 271, La Jolla, CA 92038. Admin. Rep. LJ-83-04C.

[2]Heath, C., and J. Francis. 1984. Results of research on California sea lions, San Nicholas Island, 1983. U.S. Dep. Commer., NOAA Natl. Mar. Fish. Serv., Southwest Fish. Sci. Center, P.O. Box 271, La Jolla, CA 92038. Admin. Rep. LJ-84-41C.

have been suggested as agents of premature pupping in wild populations (Delong et al., 1973; Odell, 1970, 1981), the high rate of stillbirth and non-viability in captivity may be influenced by the level of surveillance inherent to captive populations.

Male California sea lions were reported to be 3.37 cm longer and 1.41 kg heavier than female pups at birth (Le Boeuf et al., 1983). Year-to-year differences in mean birth lengths and weights have been related to differences in the mean birth date of harbor seals (Boulva, 1975). No such differences were found between the birth timing of male and female pups in the present study. Hence, discrepancy in birth size is likely due to greater growth velocity of fetal males.

Northern sea lion

Only limited data from two locations were available for captive northern sea lions. Although these animals appeared to have a temporal pattern of birth similar to, but 30 days later than, that of the closely related California sea lion, no significant latitudinal trend was found.

The northern sea lion has a wide distribution of breeding (see Fig. 1 in Loughlin et al., 1984), but little evidence exists for latitudinal variation in birth timing in the wild. For example, a review of median birth dates at rookeries from 37°N to 60°N by Merrick (1987) failed to demonstrate any latitudinal variation in this species.

Northern fur seal

A high rate of stillbirth was noted for the fur seal. This phenomenon was previously reported by Bigg (1984) during studies indicating that contact with substrate (e.g., arrival on shore) may stimulate parturition. Captive environments, with shallow pools and access to platforms, cannot adequately reproduce the pelagic environment which female fur seals inhabit during most of active gestation. Hence, premature parturition may be induced by enclosures.

The mean date of pupping for viable pups in captivity of 10 July was the same as that derived for St. George Island, Alaska (Temte, 1985). Unlike wild northern fur seals, however, the captives demonstrated no significant latitudinal variation. The sample set may have been too small to detect the estimated 0.6 days/°latitude trend reported by Temte (1985).

Pacific harbor seal

Captive harbor seals had a high rate of viable birthing with pups born over a pupping season comparable to that of wild counterparts (Temte et al., 1991). This species demonstrated stronger latitudinal effect than that of any other North Pacific pinniped covered in this report, with an average shift of 4.1 days/°latitude. Whereas this relationship is similar to that previously described for wild Pacific harbor seals on the North American west coast south of 47°N, the larger captive data set allows better definition of a curvilinear relationship.

Temte et al. (1991) separated the Pacific harbor seal into three subgroups based on birth timing. The northernmost group, from northern British Columbia and Alaska has no latitudinal variation in birth timing. The late-birthing group inhabits Puget Sound, Washington, and pups two months later than coastal seals at the same latitudes. The southern group, as noted above, has highly significant latitudinal variation. Morphometric analysis of skulls from each of these populations supports the hypothesis of discrete populations (Temte, unpubl. data). No individuals from populations originating in Puget Sound, northern British Columbia or Alaska were included in this study.

The sex of the pup, maternal age, and the previous maternal cycle (pregnant vs. non-pregnant) had no effect on birth timing. These findings are from captive animals with dependable food supplies. In contrast, Boyd (1984) has suggested that maternal condition affects implantation timing in grey seals.

Latitudinal variation and photoperiod

For captive California sea lions, a smooth and continual temporal change of pupping dates across latitude occurred despite wide diversity of climatic conditions. Nevertheless, as almost all the captives could be traced to a single wild population on the California Channel Islands, a strong environmental component appears to influence birth timing. This pattern is highly suggestive of a response to a predictable, latitudinally dependent seasonal cue. The 6-month shift in birth timing between hemispheres, as demonstrated by California sea lions in New Zealand, strongly supports a photoperiod hypothesis. Furthermore, the decreasing variance to the north (in the Northern Hemisphere) is as expected if photoperiodism occurs (Bronson, 1985). At higher latitudes, organisms experience a greater rate of change in photoperiod. Consequently, responses to specific cues should occur over a compressed time period.

Latitudinal variation in birth timing of the northern fur seal, a species with delayed implantation (Daniel, 1981), can be explained by a response to photoperiod occurring between ovulation and implantation (Temte, 1985). This sets the time of implantation and birth while allowing flexibility in estrus timing. For example, arrival at breeding grounds and mating occur as much

as two months earlier for parous cows than for virgin northern fur seals (Craig, 1964; Bigg, 1986); this disparity is not reflected in birth timing (Trites, 1992). Gentry (1981) noted, however, that individual parous females have fairly rigid year-to-year estrus timing, and Bigg (1984) has suggested that the breeding synchrony found in this species may be due to arrival on shore and on social factors.

The harbor seal has been shown to have delayed implantation (Fisher, 1954; Bigg, 1969b) and the California and northern sea lions are thought to have this reproductive pattern as well (Odell, 1975; Boshier, 1981; Schusterman, 1981). Hence, a similar mechanism may exist to signal implantation in these species. Temte (1985) proposed that northern fur seals responded to a photoperiod of 12.5 hours per day (prior to the autumnal equinox), thus explaining the positive slope in latitudinal variation. Likewise, the Pacific harbor seals in this study have a positive slope and may respond to long (>12.0 h/day) photoperiods. In contrast, California sea lions have a negative slope in latitudinal variation, which suggests a response to a photoperiod slightly less than 12.0 hours per day (following the autumnal equinox), and occurring prior to implantation.

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Variations on a simple dynamic pool model

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Abstract.—A simple dynamic pool model (the "base model"), defined by a linear weight-at-age relationship and a Cushing (convex power) stock-recruitment relationship, results in an explicit solution for the fishing mortality rate corresponding to maximum sustainable yield F_{MSY}. This solution's sensitivity can be examined by comparing it to solutions derived under alternative model specifications. Four such modifications are considered here: 1) replacing the Cushing stock-recruitment equation with an equation of the Beverton-Holt form; 2) generalizing from linear growth to a flexible form of von Bertalanffy growth; 3) allowing the ages of recruitment to the fishery a_f and the mature stock a_m to diverge; and 4) allowing for a finite maximum age in the stock. Exact polynomial solutions for F_{MSY} are derived for each specification (except the fourth), and the potential bias introduced by use of the base model is examined for each. In all cases, the solution to the base model is within 10% of the solution to the alternative model under a range of parameter values.

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Some fishery models can be solved analytically (i.e., by mathematical manipulation of the equations), while others can be solved only numerically (i.e., by the brute force of computer simulation). One advantage of analytic models is that the generality of their solutions is more straightforwardly addressed. For example, it is easy to show that the stock size associated with maximum sustainable yield (MSY) in a Schaefer (1954) model is always one-half the pristine stock size; this property follows directly from the assumption of logistic growth upon which the model is based. Such generality is more difficult to demonstrate for simulation models, however. For instance, if a particular simulation showed that MSY was obtained at a stock size equal to one-half the pristine level, there would be no way to tell whether this result was general, except by repeated trial and error with different values for the input parameters.

Another example of an analytic solution is the one obtained by Thompson (1992) for the fishing mortality rate at MSY (F_{MSY}) in his simple dynamic pool model. Because this solution is an analytic one, it is completely general in the sense that it will hold whenever the underlying assumptions of the model hold, regardless of parameter values. Of course, the underlying assumptions may *not* hold in a particular instance, which raises the question: How sensitive is the solution to those assumptions? The purpose of this paper is thus to examine the sensitivity of Thompson's (1992) solution relative to the underlying assumptions of that model. This will be accomplished by developing four reasonable modifications to the base model proposed by Thompson and by examining the range of errors that might likely be encountered if the base model were employed in situations where one of the modifications would have been more appropriate.

Review of the base model

Thompson (1992, see also Jensen, 1973) defined a simple dynamic pool model as one that reflects the following assumptions: 1) cohort dynamics are of continuous-time form; 2) vital rates are constant with respect to time and age; 3) fish mature and recruit to the fishery continuously and at the same invariant ("knife-edge") age; 4) mean body weight at age is determined by age alone; 5) the stock (or population) consists of the pool of recruited individuals; 6) maximum age is infinite; 7) the stock is in an equilibrium state determined by the fishing mortality rate; and 8) recruitment is determined by stock biomass alone. Within the framework provided by these assumptions, particular models are distinguished by the forms assigned to the weight-at-age and stock-recruitment functions.

As an example, Thompson (1992) developed a particular simple dynamic pool model than can be solved explicitly for F_{MSY}. In terms of biomass per recruit, the model is basically

the same as that of Hulme et al. (1947), where body weight is assumed to be a linear function of age (e.g., Richards, 1969):

$$w(a) = w_r\left(\frac{a-a_r}{a_r-a_0}\right), \quad (1)$$

where a represents age, a_r is the age of recruitment, a_0 is the age intercept, $w(a)$ represents individual weight at age, and w_r is the weight at recruitment.

For a given recruitment level, stock biomass in the model is given by

$$B(F') = \left(\frac{b(F',a_r)}{M}\right)\left(\frac{1+K''+F'}{(1+F')^2}\right), \quad (2)$$

where M is the instantaneous rate of natural mortality, $F'=F/M$, $B(F')$ is the equilibrium stock biomass obtained under a relative fishing mortality rate of F', $b(F',a_r)$ is the equilibrium biomass at $a=a_r$ obtained under a relative fishing mortality rate of F', and

$$K'' = \frac{1}{M(a_r-a_0)}, \quad (3)$$

which can be interpreted in this model as the pristine ratio of growth to recruitment (Thompson, 1992).

Thompson (1992) extended the model described in Equation 2 by incorporating a stock-recruitment relationship of the convex power form suggested by Cushing (1971):

$$b(F',a_r) = pB(F')^q, \quad (4)$$

where p and q are constants and $0 \le q \le 1$. In the limiting case of $q=0$, recruitment is constant, while in the other limiting case of $q=1$, recruitment is proportional to biomass.

Substituting Equation 4 into Equation 2 and rearranging terms gives the following equation for equilibrium stock biomass:

$$B(F') = \left[\left(\frac{p}{M}\right)\left(\frac{1+K''+F'}{(1+F')^2}\right)\right]^{\frac{1}{1-q}}. \quad (5)$$

Multiplying both sides of Equation 5 by MF' then gives the equation for sustainable yield, which is maximized at

$$F'_{MSY} = \frac{-(q+1)K''+1+\sqrt{(q+1)^2K''^2+(6q-2)K''+1}}{2q} - 1, \quad (6)$$

where $F'_{MSY} = F_{MSY}/M$.

In the special case where $q=0$, Equation 6 reduces to the solution for F'_{max} ($=F_{max}/M$):

$$F'_{max} = \frac{K''+1}{K''-1}. \quad (7)$$

A common rule of thumb is that F'_{MSY} should equal 1 (Alverson and Pereyra, 1969; Thompson, 1992). The locus of parameter values for which this rule holds precisely is given by

$$q = \frac{1}{K''+2}. \quad (8)$$

Another behavior of interest is the ratio of $B(F'_{MSY})$ to $B(0)$. Here, this ratio has a lower limit of $(K''-1)/(4K'')$ (at $q=0$) and an upper limit of $1/e$ (at $q=1$).

The base model presented in Equations 1–6 can be modified in a number of ways. Although such modifications may make it more difficult to obtain an explicit solution for F_{MSY}, they may also provide some guidance as to the generality of the base model's behavior. Four modifications will be considered here: 1) replacing the Cushing stock-recruitment equation with an equation of the form suggested by Beverton and Holt (1957); 2) generalizing from linear growth to a flexible form of von Bertalanffy (1938) growth; 3) allowing the ages of recruitment to the fishery a_f and the mature stock a_m to diverge; and 4) allowing for a finite maximum age in the stock. For each modification (except the fourth), polynomial solutions for F'_{MSY}, F'_{max}, and the locus at which $F'_{MSY} = 1$ will be derived, and the upper limit to the ratio $B(F'_{MSY})/B(0)$ will be presented. The potential bias introduced by use of the base model will also be examined graphically for each modification.

Beverton-Holt recruitment

The choice of stock-recruitment relationship can have an appreciable impact on the resulting estimate of F_{MSY} (Kimura, 1988). For comparative purposes, the stock-recruitment relationship of Beverton and Holt (1957) can be substituted for the Cushing form used in the base model. It will prove convenient to parametrize the Beverton-Holt equation as follows:

$$b(F',a_r) = \frac{QB(F')}{PB(F')+1}, \quad (9)$$

where Q represents the slope of the curve at the origin and P represents the ratio between the slope at the origin and the height of the asymptote.

Substituting Equation 9 into Equation 2 and solving for $B(F')$ gives

$$B(F') = \left(\frac{1}{P}\right)\left(\frac{Q(1+K''+F')}{M(1+F')^2} - 1\right), \quad (10)$$

which is the analogue to Equation 5 for the Beverton-Holt recruitment case.

Polynomial solution

Multiplying Equation 10 through by MF', differentiating with respect to F', and setting the resulting expression equal to zero gives the following polynomial solution for F'_{MSY}:

$$F'^{3}_{MSY}+3F'^{2}_{MSY}+[3+(K''-1)Q']F'_{MSY}+1-(K''+1)Q'=0, \quad (11)$$

where $Q' = Q/M$.

It is possible to solve Equation 11 explicitly for F'_{MSY}. For the case where $K''>1$,

$$F'_{MSY}=\left[Q'\left(\sqrt{K''^2+Q'\left(\frac{K''-1}{3}\right)^3}+K''\right)\right]^{1/3}- \quad (12)$$

$$\left[Q'\left(\sqrt{K''^2+Q'\left(\frac{K''-1}{3}\right)^3}-K''\right)\right]^{1/3}-1.$$

For the case where $K''=1$,

$$F'_{MSY}=(2Q')^{1/3}-1. \quad (13)$$

For the case where $K''<1$,

$$F'_{MSY}=2\sqrt{\frac{(1-K'')Q'}{3}}\cos\left(\left(\frac{1}{3}\right)\cos^{-1}\left[\left(\frac{3K''}{1-K''}\right)\sqrt{\frac{3}{(1-K'')Q'}}\right]\right)-1. \quad (14)$$

The parameter Q' functions inversely to q in the sense that Equations 11–14 reduce to Equation 7 as Q' approaches infinity (the F'_{max} case), whereas Equation 6 does so as q approaches zero. As Q' increases, F'_{MSY} increases monotonically, whereas F'_{MSY} decreases with increasing q in the base model.

As with the base model, Equations 11–14 contain $F'_{MSY}=1$ as a special case. Here this is obtained when $Q'=4$ or when K'' approaches infinity. This contrasts somewhat with the base model, where keeping F'_{MSY} at a constant value of 1.0 required an inverse relationship between q and K''. However, it should be pointed out that $F'_{MSY}=1$ is a very special case in the Beverton-Holt form of the model, since this turns out to be the only constant value of F'_{MSY} that does not imply some sort of relationship between Q' and K''. In fact, a direct relationship between Q' and K'' is required for all constant values of $F'_{MSY}>1$, as described below:

$$Q'=\frac{(F'_{MSY}+1)^3}{F'_{MSY}+1-(F'_{MSY}-1)K''}. \quad (15)$$

Another difference between this model and the base model is that here F'_{MSY} reaches zero at $Q'=1/(K''+1)$, whereas F'_{MSY} in the base model does not reach zero until $q=1$. Still another difference is that here the upper limit to the ratio $B(F'_{MSY})/B(0)$ is 0.5, contrasted with $1/e$ in the base model. In both models, this limit is reached as F'_{MSY} approaches zero.

Finally, the behavior of this modification differs from that of the base model in that extinction is possible here, owing to the Beverton-Holt curve's finite slope at the origin. Extinction occurs here at

$$F'_{ext}=\frac{Q'+\sqrt{(4K''+Q')Q'}}{2}-1. \quad (16)$$

The relative fishing mortality rate described by Equation 16 need not be unrealistically high. For example, it will be less than F'_{max} whenever the following relationship holds:

$$Q'<\frac{4K''}{K''^2-1}. \quad (17)$$

Bias resulting from the assumption of Cushing recruitment

Assuming that the stock-recruitment relationship follows the Cushing form when it actually follows a Beverton-Holt form can lead to a biased estimate of F_{MSY}. To compare stock-recruitment curves, Kimura (1988) observed that a two-parameter function can be defined by any two points on the curve. In his example, Kimura used hypothetical stock-recruitment "observations" at the pristine biomass level and at one-half the pristine biomass level. Kimura conjectured that recruitment might be reduced to about 90% of the pristine level when biomass has been reduced by 50% relative to its own pristine level, a suggestion which has been endorsed by others (e.g., Clark, 1991). Given the other parameters used in his example, Kimura found that the F_{MSY} value under a Beverton-Holt stock-recruitment relationship was much less than the value under a Cushing relationship fit to the same two stock-recruitment points.

However, there is no reason to believe *a priori* that a Cushing relationship is necessarily less conservative than a Beverton-Holt relationship in terms of its associated F_{MSY} value. Note that Equation 6 can be solved explicitly for q as a function of K'' and F'_{MSY} as follows:

$$q=\frac{K''+1-(K''-1)F'_{MSY}}{(K''+1+F'_{MSY})(F'_{MSY}+1)}. \quad (18)$$

Substituting Equation 12, 13, or 14 into Equation 18 thus gives the q value that sets F_{MSY} under a Cushing stock-recruitment relationship equal to F_{MSY} under a Beverton-Holt relationship. Assuming that both stock-recruitment curves are parametrized to pass through the same pristine stock-recruitment point $(B(0),b(0,a_r))$, this q value implies a second intersection at some lower biomass level. It turns out that this lower level is always less than about 20% of $B(0)$ (Fig. 1A) and greater than about 20% of $b(0,a_r)$ (Fig. 1B). In other words, Kimura's (1988) placement of a lower intersection at 50% of $B(0)$ would always cause the Cushing model to overestimate F_{MSY}. Placing the lower intersection at a biomass level less than 20% of $B(0)$, however, might result in either an over- or underestimate.

For example, one rule of thumb (Clark, 1991) holds that $F_{0.1}$ (the fishing mortality rate that reduces the slope of the yield-per-recruit curve to one-tenth of the slope at the origin), $F_{35\%}$ (the fishing mortality rate that reduces the level of spawning biomass per recruit to 35% of the pristine level) and M should be approximately equal. In the base model, this rule of thumb holds exactly at K''=1.5 (Thompson, in press). In the base model with Beverton-Holt recruitment, then, F_{MSY}=$F_{0.1}$=$F_{35\%}$=M at Q'=4 and K''=1.5. These parameters imply a stock-recruitment curve in which recruitment is reduced from $b(0,a_r)$ by exactly 1/11 when biomass is reduced to 50% of $B(0)$, and in which recruitment is reduced from $b(0,a_r)$ by exactly 50% when biomass is reduced to 1/11 of $B(0)$.

In the base model with Cushing recruitment, on the other hand, F_{MSY}=$F_{0.1}$=$F_{35\%}$=M at q=2/7 and K''=1.5, implying a stock-recruitment curve in which recruitment is reduced from $b(0,a_r)$ by about 18% when biomass is reduced to 50% of $B(0)$, and in which recruitment is reduced from $b(0,a_r)$ by about 50% when biomass is reduced to 1/11 of $B(0)$. Thus, in the "rule of thumb" case, the form of the stock-recruitment curve (Cushing or Beverton-Holt) has virtually no impact on the resulting estimate of F_{MSY} so long as the curve passes through $(B(0),b(0,a_r))$ and $(B(0)/11,b(0,a_r)/2)$.

More generally, to cause Cushing and Beverton-Holt curves to intersect at $(B(0),b(0,a_r))$ and at some fraction ρ of $B(0)$, set

$$q = 1 - \frac{\ln(\rho[(K''+1)Q'-1]+1) - \ln(Q') - \ln(K''+1)}{\ln(\rho)} \quad (19)$$

and

$$p = \left(\frac{M}{K''+1}\right)\left(\frac{(K''+1)Q'-1}{P}\right)^{1-q}. \quad (20)$$

Alternatively, the lower intersection can also be defined in terms of relative recruitment (as opposed to relative biomass). To cause Cushing and Beverton-Holt curves to intersect at $(B(0),b(0,a_r))$ and at some fraction θ of $b(0,a_r)$, set

$$q = 1 - \frac{\ln(\theta)}{\ln(\theta) - \ln[(1-\theta)(K''+1)Q' + \theta]} \quad (21)$$

and p as in Equation 20.

Biases resulting from placement of the lower intersection at 50% and 10% of $B(0)$ are compared in Figure 2, A and B, respectively. Note that use of the 50% value (as in Kimura's [1988] example) causes large and uniformly positive biases in the base model's estimate of F_{MSY}. On the other hand, use of the 10% value constrains bias to the +/– 10% range over a large portion of parameter space.

Biases resulting from placement of the lower intersection at 90% and 50% of $b(0,a_r)$ are compared in Figure 2, C and D, respectively (the 50% reference point has been suggested by Mace[1] and Myers et al.[2]). As with the relative biomass reference level, use of Kimura's (1988) relative recruitment reference level (90%) causes large and uniformly positive biases in the base model's estimate of F_{MSY} over a large portion of parameter space. On the other hand, use of the 50% value constrains bias to the +/– 10% range over a sizable region.

As Figure 2 illustrates, then, there is reason to believe that the form of the stock-recruitment curve (Cushing or Beverton-Holt) may not be particularly important in terms of the resulting estimate of F_{MSY} so long as the candidate curves intersect at a fairly low level. In other words, fishery managers need not always view an estimate of F_{MSY} as being critically dependent on the form of the stock-recruitment curve.

A general growth function

The linear growth function used by Thompson (1992) may be viewed as a special case of the following more

[1]Mace, P. M. 1993. Relationships between common biological reference points used as thresholds and targets of fisheries management strategies. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., 1335 East-West Highway, Silver Spring, MD 20910.

[2]Myers, R. A., A. A. Rosenberg, P. Mace, N. Barrowman, and V. Restrepo. 1993. In search of thresholds for recruitment overfishing. Dep. Fisheries and Oceans, St. John's, New Foundland. Unpubl. manuscr., 14 p.

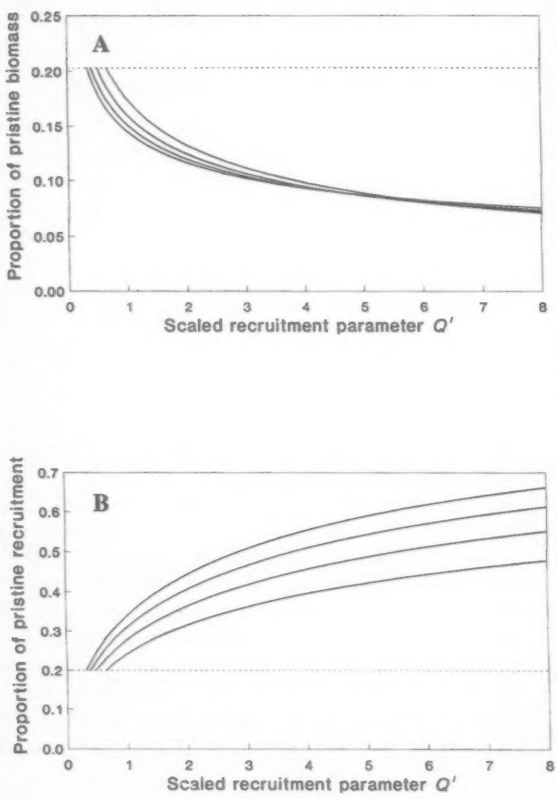

Figure 1

Location of the zero-bias intersection of the Cushing and Beverton-Holt stock-recruitment curves. Horizontal dashed lines represent limiting values, obtained in the limit as F_{MSY} goes to zero. Proceeding from left to right along a given horizontal dashed line, the curves which intersect the line correspond to K'' values of 2.0, 1.5, 1.0, and 0.5, respectively. Given values of the composite parameter K'' and the scaled Beverton-Holt recruitment parameter Q', fixing the lower intersection of the recruitment curves at the point identified by the appropriate locus in this figure causes the base model to give the same value for F'_{MSY} as the Beverton-Holt modification. (**A**) Zero-bias intersection defined in terms of relative stock biomass. Points below and to the left of the curves result in negative bias, while points above and to the right of the curves result in positive bias. (**B**) Zero-bias intersection defined in terms of relative recruitment. Points below and to the right of the curves result in negative bias, while points above and to the left of the curves result in positive bias.

general form:

$$w(a) = w_r\left(\frac{1-e^{-K(a-a_r)-K'/K''}}{1-e^{-K'/K''}}\right)^n, \qquad (22)$$

where K is Brody's growth coefficient, $K' = K/M$, and n is a positive integer. In different parametrizations, Equation 22 corresponds to (or is a special case of) a number of general growth functions, including those of Richards (1959, see also Fletcher, 1975), Savageau (1980), and Schnute (1981). Schnute described his parametrization of Equation 22 as "generalized von Bertalanffy growth" (although he did not restrict n to integer values). When n=3, Equation 22 corresponds to the common ("specialized") von Bertalanffy (1938) curve, and, when n=1, the "monomolecular" curve of Pütter (1920) and Brody (1928) is obtained.

In the limit as K approaches zero, Equation 22 gives an nth-degree polynomial in age:

$$w(a) = w_r\left(\frac{a-a_0}{a_r-a_0}\right)^n, \qquad (23)$$

which has been used to describe growth (though not always in weight) by Mendelsohn (1963), Dethlefsen et al. (1968), Knight (1968), Rafail (1972), Roff (1980), Geoghegan and Chittenden (1982), Standard and Chittenden (1984), and Chen et al. (1992). Equation 1 thus represents the special case of Equation 22 where K approaches zero and n=1.

Polynomial solution

The polynomial solution for this model is partitioned into two cases (K=0 and K>0) and derived in the Appendix.

When K=0, the solution for F'_{max} can be written as a polynomial of degree n, and the solution for F'_{MSY} can be written as a polynomial of degree n+1. When K>0, the solution for F'_{max} can be written as a polynomial of degree $2n$, and the solution for F'_{MSY} can be written as a polynomial of degree $2n$+1. As with the base model, the solution in either case indicates that maintaining an F'_{MSY} value of 1.0 requires an inverse relationship between q and K'' (which, as in the base model, can be written explicitly). Likewise, the upper limit to the ratio $B(F'_{MSY})/B(0)$ is the same as in the base model ($1/e$) in both cases.

The polynomial solution can be manipulated easily to show how it varies across the range of possible K', q, and K'' values. For example, several limiting values of F'_{max} and F'_{MSY} are shown in Table 1.

Bias resulting from the assumption of linear growth

Assuming that growth is linear when it actually follows a generalized von Bertalanffy form can lead to a

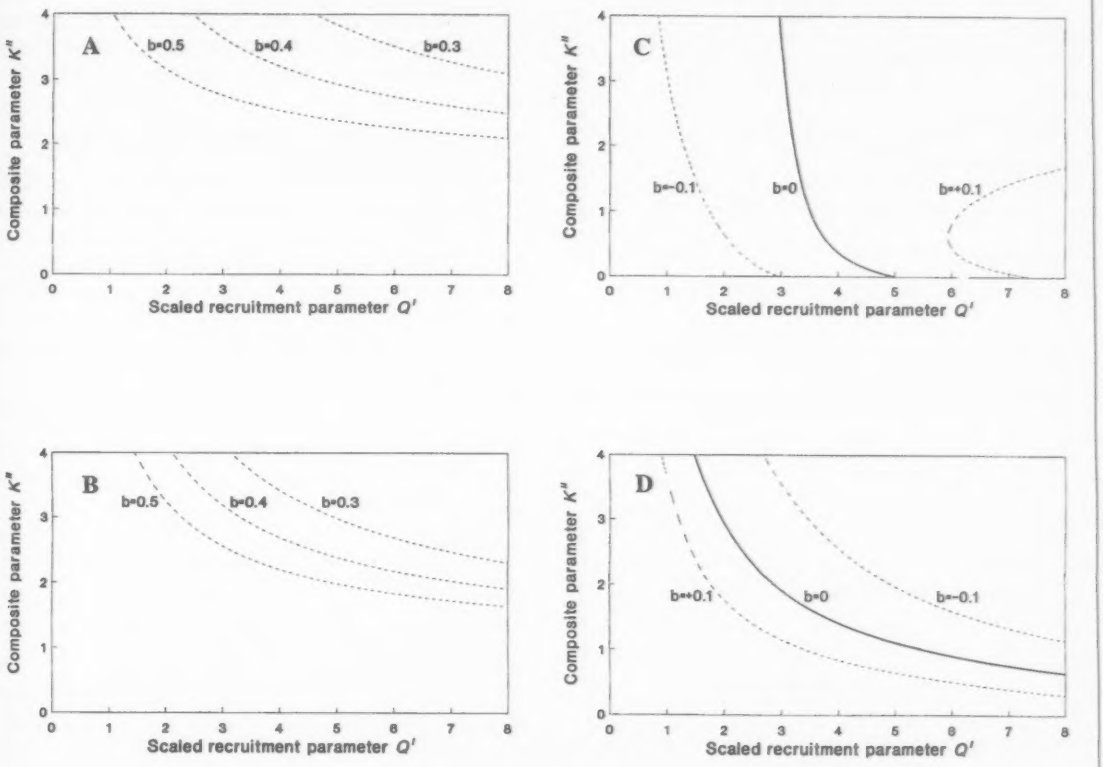

Figure 2

Isobias loci obtained when the base model is used to approximate the Beverton-Holt modification under four calibration methods. (**A**) Isobias loci obtained when the lower intersection of the Cushing and Beverton-Holt stock-recruitment curves is fixed at 50% of pristine biomass. Loci corresponding to biases in F'_{MSY} of 30% (b=0.3), 40% (b=0.4), and 50% (b=0.5) are shown. (**B**) Isobias loci obtained when the lower intersection of the Cushing and Beverton-Holt stock-recruitment curves is fixed at 10% of pristine biomass. Loci corresponding to biases in F'_{MSY} of −10% (b=–0.1), 0% (b=0), and +10% (b=+0.1) are shown. (**C**) Isobias loci obtained when the lower intersection of the Cushing and Beverton-Holt stock-recruitment curves is fixed at 90% of pristine recruitment. Loci corresponding to biases in F'_{MSY} of 30% (b=0.3), 40% (b=0.4), and 50% (b=0.5) are shown. (**D**) Isobias loci obtained when the lower intersection of the Cushing and Beverton-Holt stock-recruitment curves is fixed at 50% of pristine recruitment. Loci corresponding to biases in F'_{MSY} of +10% (b=+0.1), 0% (b=0), and −10% (b=–0.1) are shown.

biased estimate of F_{MSY}. One way to compare the two types of curve is to require that they intersect at w_r and that they imply the same pristine biomass-per-recruit level. In the base model, stock biomass per recruit is obtained by multiplying Equation 2 through by $w_r/b(F',a_r)$. When Equation 22 or 23 is used to represent growth, stock biomass per recruit is given by Appendix Equation 5 or Appendix Equation 11. When growth curves are forced to intersect at w_r and pristine biomass per recruit levels are equated, the following parametrization is defined:

$$\hat{K}'' = (1 - e^{-K'/K''})^{-n}\left(\sum_{k=0}^{n} \frac{(-1)^k \binom{n}{k} e^{-kK'/K''}}{kK' + 1}\right) - 1, \qquad (24)$$

where $\binom{n}{k}$ is the binomial coefficient (Appendix) and $\hat{K}''$ is the estimated value of K'' used to define the linear growth relationship (assuming that M and a_r are the same under both weight-at-age relationships, $\hat{K}''$ is distinguished from K'' by the fact that the age intercepts of the two curves differ—Equation 3).

Substituting Equation 24 for K'' in Equation 6 gives F'_{MSY} in the base model when the linear growth function is fit in the manner described above. This F'_{MSY} value can be either higher or lower than the value given by the solution derived in the Appendix. For the case where n=3, Figure 3 shows the range of +/– 10% bias for four values of K'', along with the loci of zero bias. Note that at low values of K'' (e.g., 0.5), the two

Table 1
Limits on F'_{max} and F'_{MSY} under generalized von Bertalanffy growth.

	$K'=0$	$K'\to\infty$
Limits on F'_{max}		
$K''\to\infty$	$F'_{max}=\frac{1}{n}$	$F'_{max}\to\infty$
$F'_{max}\to\infty$	$K''=\lim_{K'\to 0}\left(\frac{K'}{\ln(nK'+1)}\right)=\frac{1}{n}$	$K''\to\infty$
Limits on F'_{MSY}		
$K''\to\infty$	$F'_{MSY}=\frac{1-q}{n+q}$	$F'_{MSY}=\frac{1-q}{q}$
$K''=0$	$F'_{MSY}=\frac{1-q}{q}$	$F'_{MSY}=\frac{1-q}{q}$

F'_{MSY} values tend to be close over a large portion of parameter space, but that as K'' increases, the base model's estimate of F'_{MSY} is more likely to be significantly biased.

Divergent ages of recruitment and maturity

As defined by Thompson (1992), all simple dynamic pool models assume that fish recruit to both the fishery and the mature stock at a single age a_r. However, in more complicated dynamic pool models, it is common for the age (or size) of recruitment to the fishery to be treated as a management variable.

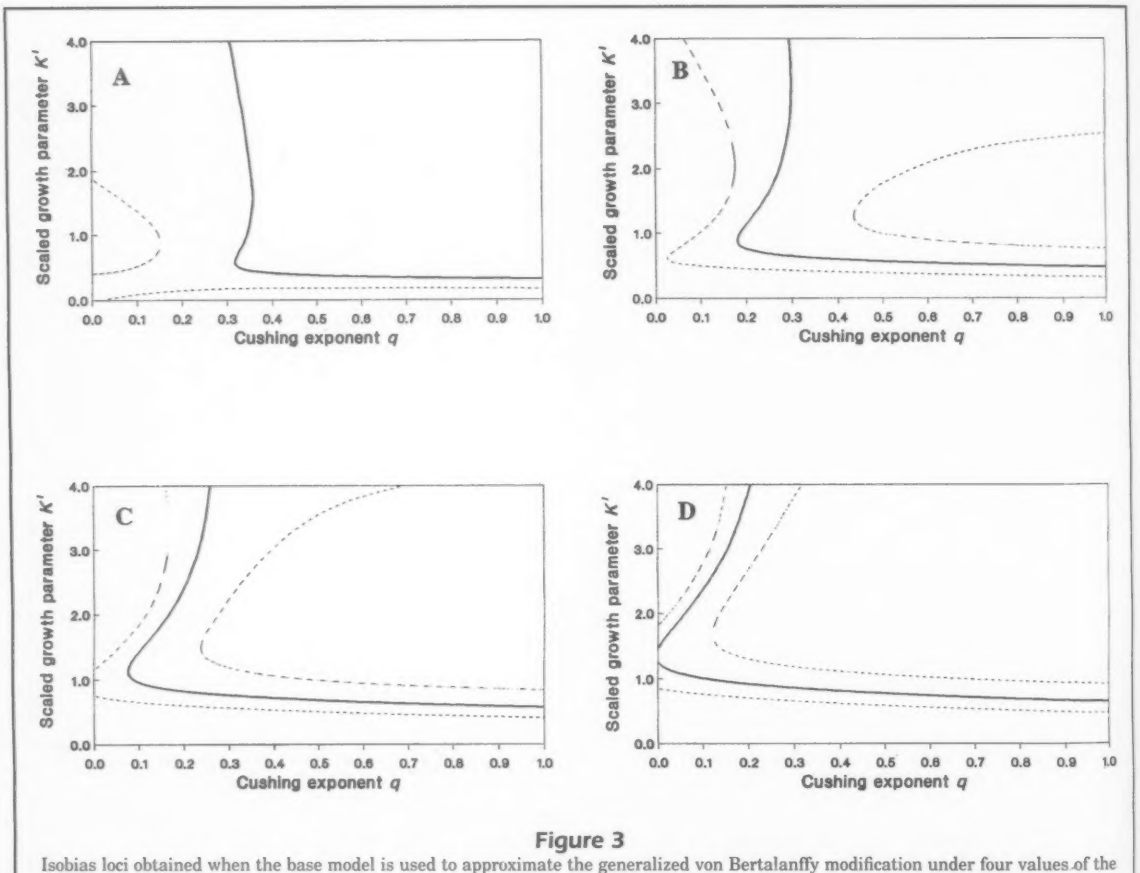

Figure 3
Isobias loci obtained when the base model is used to approximate the generalized von Bertalanffy modification under four values of the composite parameter K(n=3 in all cases). Solid curves indicate loci of zero bias in F'_{MSY}. The dashed curve to the left of the solid curve in each panel indicates the locus of +10% bias, while the dashed curve on the right indicates the locus of −10% bias. (**A**) Isobias loci for K''=0.5. (**B**) Isobias loci for K''=1.0. (**C**) Isobias loci for K''=1.5. (**D**) Isobias loci for K''=2.0.

To accommodate this change, the Cushing stock-recruitment relationship (Equation 4) has to be re-written to specify that only the mature biomass $B_m(F')$ contributes to recruitment:

$$b(F',a_f) = p\,B_m(F')^q. \tag{25}$$

Equation 2 can be rewritten to express total fishable biomass as

$$B_f(F') + \left(\frac{b(F',a_f)}{M}\right)\left(\frac{1 + K_f'' + F'}{(1+F')^2}\right), \tag{26}$$

where $K_f'' = 1/[M(a_f - a_0)]$, after Equation 3.

Polynomial solution

Age at recruitment exceeds age at maturity In the case where a_f exceeds a_m, recruitment to the fishery and to the mature stock are related as follows:

$$b(F',a_f) = b(F',a_m)\left(\frac{w(a_f)}{w(a_m)}\right) e^{-M(a_f - a_m)} =$$
$$b(F',a_m)\left(\frac{K_m''}{K_f''}\right) e^{-\left(\frac{1}{K_f''} - \frac{1}{K_m''}\right)}, \tag{27}$$

where $K_m'' = 1/[M(a_m - a_0)]$, after Equation 3.

Total mature biomass can then be expressed as

$$B_m(F') = \left(\frac{b(F',a_m)}{a_m - a_0}\right)\int_{a_m}^{a_f}(a - a_0)e^{-M(a-a_m)}da + B_f(F')$$

$$= \left(\frac{b(F',a_m)}{M}\right)\left[\left(1 + \frac{1}{M(a_m - a_0)}\right)\right.$$

$$\left. - e^{-M(a_f - a_m)}\left(\frac{a_f - a_0}{a_m - a_0} + \frac{1}{M(a_m - a_0)}\right)\right] + B_f(F') \tag{28}$$

$$= \left(\frac{b(F',a_m)}{M}\right)\left[1 + K_m'' - e^{-\left(\frac{1}{K_f''} - \frac{1}{K_m''}\right)}\left(\frac{K_m''}{K_f''} + K_m''\right)\right] + B_f(F')$$

$$= \frac{b(F',a_m)\,(1 + K_m'') - b(F',a_f)\,(1 + K_f'')}{M} + B_f(F').$$

Equations 25–28 constitute a set of four equations in four unknowns [$b(F',a_f)$, $b(F',a_m)$, $B_f'(F')$, and $B_m(F')$]. Solving simultaneously gives

$$B_f(F') = \left(\frac{p}{M}\right)\left(\frac{1+K_f''+F'}{(1+F')^2}\right)\left[\left(\frac{p}{M}\right)\left(\frac{1+K_m''+F'}{(1+F')^2}\right) + \alpha(K_f'')\right]^{\frac{q}{1-q}}, \tag{29}$$

where

$$\alpha(K_f'') = \left(\frac{K_f''}{K_m''}\right)\left(\frac{1 + K_m''}{e^{-\left(\frac{1}{K_f''} - \frac{1}{K_m''}\right)}}\right) - K_f'' - 1. \tag{30}$$

Multiplying Equation 29 through by MF', differentiating with respect to F', and setting the resulting expression equal to zero gives the following polynomial solution for F'_{MSY} (which collapses to Equation 6 when $K_f'' = K_m''$):

$$\begin{aligned}&((1-q)[\alpha(K_f'')(1-K_f'')+1]-1)F'^{3}_{MSY}+\\ &((1-q)[\alpha(K_f'')(3-K_f'')+2K_f''+3]-3K_f''-2)F'^{2}_{MSY}+\\ &(1-q)[\alpha(K_f'')(3+K_f'')+K_f''^2+4K_f''+3]-2K_f''^2-3K_f''-1)F'_{MSY}+\\ &(1-q)[\alpha(K_f'')(1+K_f'')+K_f''^2+2K_f''+1] = 0.\end{aligned} \tag{31}$$

The solution for F'_{max} in this model is the same as in the base model (Equation 7).

Equation 31 contains $F'_{MSY} = 1$ as a special case, obtained when the following relationship holds:

$$q = 1 - \frac{(K_f'' + 2)\,(K_f'' + 1)}{4\alpha(K_f'') + (K_f'' + 2)^2}. \tag{32}$$

Just as the base model required an inverse relationship between q and K'' in order for F'_{MSY} to equal 1.0 (Equation 8), this model requires an inverse relationship between q and K_f''. Likewise, the upper limit to the ratio $B(F_{MSY})/B(0)$ is the same as in the base model ($1/e$).

Age at maturity exceeds age at recruitment Another possible modification is to allow a_m to exceed a_f. This requires rewriting Equation 27 as follows:

$$b(F',a_f) = b(F',a_m)\left(\frac{K_m''}{K_f''}\right) e^{-(1+F')\left(\frac{1}{K_f''} - \frac{1}{K_m''}\right)}. \tag{33}$$

The previous expressions for recruitment (Equation 25) and equilibrium fishable biomass (Equation 26) can be applied without modification. However, because the entire mature stock is subject to both fishing and natural mortality, the previous expression for equilibrium mature biomass (Equation 28) is simplified to

$$B_m(F') = \left(\frac{b(F',a_m)}{M}\right)\left(\frac{1 + K_m'' + F'}{(1+F')^2}\right). \tag{34}$$

Solving Equations 25, 26, 33, and 34 simultaneously gives

$$B_f(F') = \left(\frac{p}{M}\right)\left(\frac{1+K''_f+F'}{(1+F')^2}\right)\left[\left(\frac{p}{M}\right)\left(\frac{1+K''_m+F'}{(1+F')^2}\right)\times \left(\frac{K''_f}{K''_m}\right)e^{-(1+F')\left(\frac{1}{K''_m}-\frac{1}{K''_f}\right)}\right]^{\frac{q}{1-q}}. \quad (35)$$

The fact that F' appears in the exponent in Equation 35 complicates the solution for F'_{MSY} somewhat, increasing the degree of the polynomial solution to four:

$$\left(\frac{1}{K''_m}-\frac{1}{K''_f}\right)qF'^{4}_{MSY}+\left[(K''_f+K''_m+3)\left(\frac{1}{K''_m}-\frac{1}{K''_f}\right)+1\right]qF'^{3}_{MSY}+\left[(K''_fK''_m+2K''_f+2K''_m+3)\left(\frac{1}{K''_m}-\frac{1}{K''_f}\right)q+(2K''_m+3)q+K''_f-1\right]F'^{2}_{MSY}+\left[(K''_f+1)(K''_m+1)\left(\frac{1}{K''_m}-\frac{1}{K''_f}\right)q+(K''_f+3)(K''_m+1)q+K''_fK''_m-K''_m-2\right]F'_{MSY}-(K''_f+1)(K''_m+1)(1-q)=0. \quad (36)$$

The solution for F'_{max} is the same as in the base model (Equation 7).

Equation 36 contains $F'_{MSY}=1$ as a special case, obtained when the following relationship holds:

$$q=\frac{K''_m+2}{(K''_f+2)(K''_m+2)\left(\frac{1}{K''_m}-\frac{1}{K''_f}\right)+K''_f(K''_m+1)+3K''_m+4}. \quad (37)$$

As with the base model (Equation 8), the above expression implies an inverse relationship between q and K''_f. Likewise, the upper limit to the ratio $B(F'_{MSY})/B(0)$ remains the same ($1/e$).

Bias resulting from the assumption of $a_f=a_m$

When $a_f>a_m$, Equation 6 tends to underestimate F'_{MSY}. Loci of –10% bias are shown in Figure 4A for four values of K''_m. Parameter combinations above a particular curve and below the horizontal line $K''_f = K''_m$ result in an F'_{MSY} estimate that is within 10% of the value given by Equation 31. Note that the base model's solution is fairly sensitive to K''_f when K''_m is low. For example, when K''_m=0.5, almost any value of $K''_f<K''_m$ will result in Equation 6 underestimating F'_{MSY} by more than 10%. At higher K''_m values (e.g., K''_m>1), the base model's solution is less sensitive.

The results for the case where $a_f<a_m$ are similar, except that here Equation 6 tends to overestimate rather than underestimate F'_{MSY}. Loci of +10% bias are shown in Figure 4B for four values of K''_m. Parameter combinations below a particular curve and above the horizontal line $K''_f=K''_m$ result in an F'_{MSY} estimate that is within 10% of the value given by Equation 31. Again, the base model's solution is fairly sensitive to K''_f when K''_m is low (e.g., K''_m=0.5), while at higher values (e.g., K''_m>1), the base model's solution is less sensitive.

Finite maximum age

As defined by Thompson (1992), all simple dynamic pool models exhibit mortality and growth rates which are independent of age (above the age of recruitment). This implies that there is no maximum age. However, in more complicated dynamic pool models, it is common to specify a maximum age above which all remaining fish die in knife-edge fashion. As noted by Fletcher (1987), misspecification of maximum age can introduce significant bias into some models. When the base model is modified so as to exhibit a finite maximum age (a_{max}), Equation 5 will tend to overestimate true equilibrium stock biomass, which can be written as

$$B(F') = \left[\left(\frac{p}{M}\right)\left(\frac{K''+1+F'}{(1+F')^2}-\left(\frac{K''_{max}+1+F'}{(1+F')^2}\right)\left(\frac{K''}{K_{max}}\right)e^{-(1+F')\left(\frac{1}{K''_{max}}-\frac{1}{K''}\right)}\right)\right]^{\frac{1}{1-q}}, \quad (38)$$

where $K''_{max}=1/[M(a_{max}-a_0)]$, after Equation 3. The difference inside the exterior parentheses in Equation 38 is proportional to the difference between two calculations of stock biomass per recruit in a population with infinite maximum age, where the first calculation begins the integral (over age) at age a_r and the second begins at age a_{max}. Subtracting the second term from the first adjusts for the assumption of a maximum age at $a=a_{max}$.

Because of the presence of F' in the exponential term in Equation 38, it is not possible to solve for F'_{MSY} explicitly in this modification.

Bias resulting from the assumption of infinite maximum age

Note that as K''_{max} becomes small (e.g., as a_{max} becomes large), the proportion surviving to the maximum age (the exponential term in Equation 38) goes to zero and

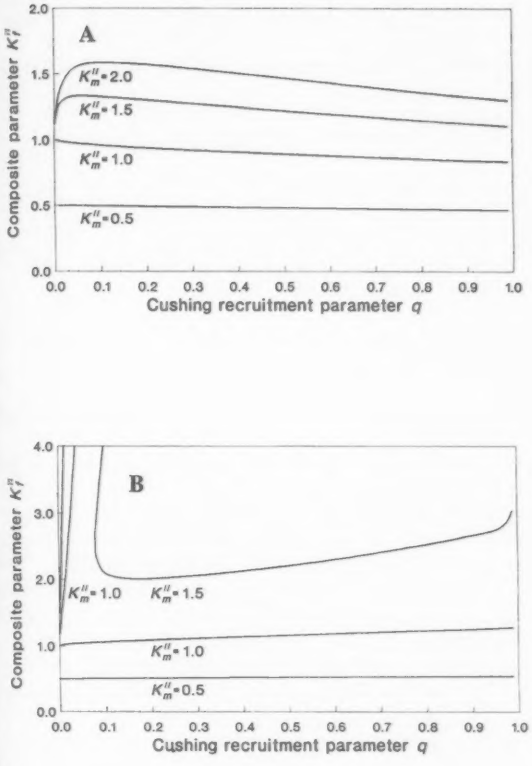

Figure 4

Isobias loci obtained when the base model is used to approximate the modification in which the ages of recruitment to the fishery and to the mature stock diverge. (**A**) Loci of –10% bias in F'_{MSY} obtained when the age of recruitment to the fishery is greater than the age of recruitment to the mature stock. (**B**) Loci of +10% bias in F'_{MSY} obtained when the age of recruitment to the fishery is less than the age of recruitment to the mature stock.

Equation 38 collapses to Equation 5. However, at any positive value of K''_{max}, Equation 5 will tend to overestimate the true value of $B(F')$ to some extent. Conversely, Equation 6 will tend to underestimate the true value of F'_{MSY}. Figure 5 shows loci of –10% bias in Equation 6's estimate of F'_{MSY}. Points above and to the left of the curves result in a bias of less than 10% (absolute value). For example, a stock with M=0.2, a_0=–1, and a_{max}=24 would have a K''_{max} value of 0.2. For such a stock, Equation 6's estimate of F'_{MSY} would be within 10% of the correct value for any value of K''>0.5 so long as q was less than about 0.53.

Conclusion

Four modifications to the base model presented by Thompson (1992) have been considered (Beverton-Holt recruitment, generalized von Bertalanffy growth, divergent ages of recruitment and maturity, and finite maximum age). The first three modifications all increase the degree of the polynomial solution for F_{MSY} (Table 2), while the fourth modification renders a polynomial solution impossible.

In order to make the Cushing stock-recruitment form of the model comparable to the Beverton-Holt form, an acceptable approximation can often be made by equating the pristine stock-recruitment points and placing the other (nonzero) intersection of the stock-recruitment curves at a fairly low level (e.g., at 10% of the pristine biomass level or at 50% of the pristine recruitment level).

In order to make the linear growth form of the model comparable to the generalized von Bertalanffy growth form, an acceptable approximation can often be made by equating the weights at recruitment and the pristine biomass-per-recruit ratios.

When the ages of recruitment to the fishery and to the mature stock diverge sufficiently or when the maximum age in the stock is sufficiently low, the base model can produce a significantly biased estimate of F_{MSY}. Except for the case in which the age of recruitment to the fishery precedes the age of recruitment to the mature stock, though, it is helpful to note that the base model always errs on the conservative side.

In conclusion, it appears that simple models (at least the base model considered here) may often perform adequately even when the true dynamics of the system follow more complicated formulae. This tends to confirm the results of studies by Silliman (1971), Roff (1983), and Ludwig and Walters (1985), who also found that simple models could perform at least as well as more complex versions in a variety of situations.

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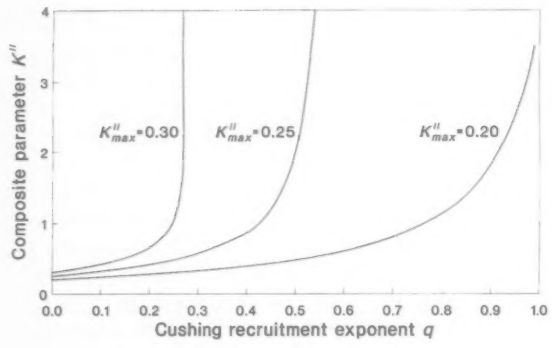

Figure 5

Loci of –10% bias in F'_{MSY} obtained when the base model is used to approximate the modification in which the maximum age in the population is finite. Points above and to the left of the curves correspond to a bias of less than 10% (absolute value), while points below and to the right of the curves correspond to a bias of greater than 10% (absolute value).

Table 2

Degree of polynomial solutions for F'_{MSY} under various modifications.

Modification	Degree
Beverton-Holt stock-recruitment	3
Generalized von Bertalanffy growth	
K=0	n+1
K>0	2n+1
Divergent ages of recruitment and maturity	
$a_f > a_m$	3
$a_f < a_m$	4

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Appendix

Some combinatoric terms

In order to incorporate Equations 22 or 23 into the model, it is helpful to define a few concepts taken from combinatorial theory (the notation used here follows Riordan [1980]). First, the number of permutations of n objects taken k at a time is given by

$$(n)_k = \frac{n!}{(n-k)!}, \tag{A1}$$

with $(n)_k$ defined as zero for $k<0$ or $k>n$.

The number of combinations (i.e., permutations without regard to order) of n objects taken k at a time is given by the binomial coefficient

$$\binom{n}{k} = \frac{(n)_k}{k!} = \frac{n!}{k!\,(n-k)!}, \tag{A2}$$

with $\binom{n}{k}$ defined as zero for $k<0$ or $k>n$.

The number of ways in which an n-element set can be partitioned into k subsets is given by Stirling numbers of the second kind, written

$$S(n,k) = \left(\frac{1}{k!}\right)\sum_{\lambda=0}^{k}(-1)^{(k-\lambda)}\binom{k}{\lambda}\lambda^n. \tag{A3}$$

The coefficients of the polynomial expansion of $(x)_n$ are given by Stirling numbers of the first kind, written

$$s(n,k) = \sum_{\lambda=0}^{n-k}(-1)^{\lambda}\binom{n-1+\lambda}{n-k+\lambda}\binom{2n-k}{n-k-\lambda}S(n-k+\lambda,\lambda). \tag{A4}$$

Polynomial solution for a generalized growth function

***K*=0** Beginning with the simpler case where K=0 (Equation 23), stock biomass per recruit can be written

$$BPR(F') = \int_{a_r}^{\infty} w_r\left(\frac{a-a_r}{a_r-a_0}\right)^n e^{-M(1+F')(a-a_r)}\,da$$
$$= \left(\frac{w_r}{M}\right)\sum_{k=0}^{n}\frac{(n)_k K'^k}{(1+F')^{k+1}}. \tag{A5}$$

In general, stock biomass in any simple dynamic pool model with Cushing recruitment can be written as the following function of biomass per recruit:

$$B(F') = \left(\frac{pBPR(F')}{w_r}\right)^{\frac{1}{1-q}}. \tag{A6}$$

Substituting Appendix Equation 5 (A5) into Appendix Equation 6 (A6), multiplying through by MF', and differentiating gives the following expression:

$$\frac{dY(F')}{dF'} = \left(\frac{pB(F')^q}{(1-q)(1+F')^{n+2}}\right)\times$$
$$\left(\sum_{k=0}^{n}[(n)_k K'^k(1-kF')(1+F')^{n-k}] - \right. \tag{A7}$$
$$\left. q\sum_{k=0}^{n}[(n)_k K'^k(1+F')^{n+1-k}]\right).$$

The solution to Equation A7 can be written as a polynomial of degree $n+1$ as follows:

$$\sum_{i=0}^{n}\left(\sum_{k=0}^{n}\left[\binom{n-k}{i}-k\binom{n-k}{i-1}\right](n)_k K'^k - \left[\sum_{k=0}^{n}\binom{n+1-k}{i}(n)_k K'^k\right]q\right)F'_{MSY}{}^i - qF'_{MSY}{}^{n+1}=0. \quad \text{(A8)}$$

In the special case where $q=0$, Equation A8 gives the following polynomial solution for F'_{max}:

$$\sum_{i=0}^{n}\left(\sum_{k=0}^{n}\left[\binom{n-k}{i}-k\binom{n-k}{i-1}(n)_k K'^k\right]\right)F'_{max}{}^i=0. \quad \text{(A9)}$$

Equation A7 can be solved explicitly for q. The locus at which $F'_{MSY}=1$ is given by

$$q=\left(\frac{1}{2}\right)\left(1-\frac{\sum_{k=0}^{n}\left[k\,(n)_k\left(\frac{K'}{2}\right)^k\right]}{\sum_{k=0}^{n}\left[(n)_k\left(\frac{K'}{2}\right)^k\right]}\right). \quad \text{(A10)}$$

Note that Equations 6–8 constitute the special cases of Equations A8–A10 where $n=1$.

***K*>0** When growth follows the form of Equation 22, stock biomass per recruit can be written

$$BPR(F')=\int_{a_r}^{\infty} w_r\left(\frac{1-e^{-K(a-a_r)-K'/K^{\infty}}}{1-e^{-K'/K^{\infty}}}\right)^n e^{-M(1+F')(a-a_r)}\,da = \left(\frac{w_r}{M(1-e^{-K'/K^{\infty}})^n}\right)\left(\sum_{k=0}^{n}\frac{(-1)^k\binom{n}{k}e^{-kK'/K^{\infty}}}{kK'+1+F'}\right). \quad \text{(A11)}$$

Substituting Equation A11 into Equation A6, multiplying through by MF', and differentiating gives the following expression:

$$\frac{dY(F')}{dF'}=MB(F')\left(1-\left(\frac{F}{(1-q)\sum_{k=0}^{n}\frac{(-1)^k\binom{n}{k}e^{-kK'/K^{\infty}}}{kK'+1+F'}}\right)\times\sum_{k=0}^{n}\frac{(-1)^k\binom{n}{k}e^{-kK'/K^{\infty}}}{(kK'+1+F')^2}\right). \quad \text{(A12)}$$

Letting

$$z(F')=\frac{MB(F')}{(1-q)\left(\sum_{k=0}^{n}\frac{(-1)^k\binom{n}{k}e^{-kK'/K^{\infty}}}{kK'+1+F'}\right)\prod_{k=0}^{n}(kK'+1+F')^2}, \quad \text{(A13)}$$

Equation A12 can be rewritten as

$$\frac{dY(F')}{dF'}=z(F')\left[\sum_{k=0}^{n}\left((-1)^k\binom{n}{k}e^{-kK'/K^{\infty}}[(1-q)\times(kK'+1)-qF']\left(\prod_{m\neq k}(mK'+1+F')^2\right)\right)\right]. \quad \text{(A14)}$$

The term enclosed in large square brackets in Equation A14 can be expanded to polynomial form. Proceeding in steps, first note that

$$\prod_{m=0}^{n-1}(mK'+1)=\sum_{m=0}^{n-1}(-1)^m s(n,n-m)\,K'^m. \quad \text{(A15)}$$

Letting

$$\alpha_{k,m}=\sum_{\lambda=0}^{m}(-1)^{m+\lambda}(n-k)_\lambda s(n-\lambda,n-m) \quad \text{(A16)}$$

(except for $\alpha_{0,n}=n!$), Equation A15 can be extended to

$$\sum_{k=0}^{n}\left(\prod_{n\neq k}(mK'+1)\right)=\sum_{k=0}^{n}\left(\sum_{m=0}^{n}\alpha_{k,m}K'^m\right). \quad \text{(A17)}$$

Next, let

$$\beta_{k,l,m}=\alpha_{k,m}\binom{n-m}{l}, \quad \text{(A18)}$$

in which case Equation A17 can be extended to

$$\sum_{k=0}^{n}\left(\prod_{m\neq k}(mK'+1+F')\right)=\sum_{k=0}^{n}\left(\sum_{l=0}^{n}\left(\sum_{m=0}^{n}\beta_{k,l,m}K'^m\right)F'^l\right). \quad \text{(A19)}$$

Then, let

$$\gamma_{i,j,k}=\sum_{l=0}^{i}\left(\sum_{m=\min(0,j-n)}^{j}\beta_{k,l,m}\beta_{k,i-l,j-m}\right), \quad \text{(A20)}$$

in which case Equation A19 can be extended to

$$\sum_{k=0}^{n}\left(\prod_{m\neq k}(mK'+1+F')^2\right)=\sum_{i=0}^{2n}\left(\sum_{j=0}^{2n-i}\left(\sum_{k=0}^{n}\gamma_{i,j,k}K'^j\right)F'^i\right). \quad \text{(A21)}$$

Finally, the solution to Equation A14 can be written as a polynomial of degree $2n+1$ as follows:

$$\sum_{i=0}^{2n}\left[\sum_{j=0}^{2n-i}\left(\sum_{k=0}^{n}(-1)^k\binom{n}{k}(k\gamma_{i,j-1,k}+\gamma_{i,j,k})e^{-kK'/K^{\infty}}\right)K'^j - \left(\sum_{j=0}^{2n-i}\left(\sum_{k=0}^{n}(-1)^k\binom{n}{k}(k\gamma_{i,j-1,k}+\gamma_{i,j,k}+\gamma_{i-1,j,k})e^{-kK'/K^{\infty}}\right)K'^j\right)q\right]F'_{MSY}{}^i-\left(\sum_{k=0}^{n}(-1)^k\binom{n}{k}e^{-kK'/K^{\infty}}\right)qF'_{MSY}{}^{2n+1}=0. \quad \text{(A22)}$$

In the special case where $q=0$, Equation A22 gives the following polynomial solution for F'_{max}:

$$\sum_{i=0}^{2n}\left[\sum_{j=0}^{2n-i}\left(\sum_{k=0}^{n}(-1)^k\binom{n}{k}(k\gamma_{i,j-1,k}+\gamma_{i,j,k})e^{-kK'/K'}\right)K'^j\right]F'^i_{max}=0. \quad \text{(A23)}$$

Equation A14 can be solved explicitly for q. The locus at which $F'_{MSY}=1$ is given by

$$q=\frac{\sum_{k=0}^{n}\left[(-1)^k\binom{n}{k}e^{-kK'/K'}\left(\prod_{m\neq k}(mK'+2)^2\right)(kK+1)\right]}{\sum_{k=0}^{n}\left[(-1)^k\binom{n}{k}e^{-kK'/K'}\left(\prod_{m\neq k}(mK'+2)^2\right)(kK+2)\right]}. \quad \text{(A24)}$$

Relationships between otolith microstructure, microchemistry, and early life history events in Dover sole, *Microstomus pacificus*

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Abstract.—Patterns of otolith microstructure and microchemistry (Sr/Ca ratios) are described in larval and juvenile Dover sole and related to developmental events and habitat. The initiation of metamorphosis is associated with a transition from clear (protein rich) to opaque (protein poor) material and formation of the first accessory primordium. Settlement is associated with the point at which growth from accessory primordia completely encloses growth from the central primordium and Sr/Ca ratio is minimal. No discrete otolith landmarks coincide with termination of metamorphosis.

Contrary to other flatfish that have a more abrupt metamorphosis, accessory primordia do not form until the left eye traverses the middorsal ridge of the cranium in Dover sole, an event that may occur months after the eye first reaches that position. A period of 70 days or more can separate the first- and last-formed accessory primordia in Dover sole, suggesting that all accessory primordia do not form in response to a single event.

A relationship between increments and days is validated for Stage 3–5 metamorphic and post-metamorphic stages. Duration of Stages 3 and 4 was in close agreement with predictions based on seasonal distributions. Duration of Stages 1 and 2, as determined by unvalidated increment counts, was about half as long as determined from seasonal distributions.

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Changes in otolith microstructure often correlate,and are presumed synchronous, with morphological and behavioral changes in larval and juvenile fish (Brothers and McFarland, 1981; Victor, 1982; Fowler, 1989; Ozawa and Penaflor, 1990; Gartner, 1991). The formation of secondary growth centers (accessory primordia) on flatfish otoliths is associated with metamorphosis in starry flounder, *Platichthys stellatus* (Campana, 1984a); plaice, *Pleuronectes platessa* (Alhossaini et al., 1989, Karakiri et al., 1989); California halibut, *Paralichthys californicus* (Kramer, 1991); and winter flounder, *Pseudopleuronectes americanus* (Sogard, 1991). Daily growth increments from accessory primordia are wider and are more likely composed of subdaily increments than those from the central primordium (Campana, 1984a; Karakiri et al., 1989; but also see Alhossaini et al., 1989).

Metamorphosis in flatfish includes a number of morphological and behavioral changes, most notably, eye migration and settlement from the water column to the sea floor. In most flatfish species, metamorphosis appears to be rapid; eye migration and settlement occur nearly simultaneously over a period of about 1 week (starry flounder, winter flounder, California halibut) to 3 weeks (plaice) (Ryland, 1966; Policansky, 1982; Campana, 1984a; Chambers and Leggett, 1987; Gadomski et al., 1992). Although timing of accessory primordium formation is not discussed in these studies, examination of published photographs suggests that the oldest and most recent accessory primordia are separated by only a few daily growth increments. In plaice, the earliest-formed accessory primordium (Alhossaini et al., 1989) or an unspecified accessory primordium (Karakiri et al., 1989) have been interpreted as settlement marks. In winter flounder, the earliest-formed accessory primordium has been interpreted as the point from which post-metamorphic age of an individual is determined (Sogard and Able, 1992). Counts and measurements of growth increments from accessory primordia to the otolith edge have been used to infer settle-

ment dates and post-settlement growth and mortality rates (Alhossaini et al., 1989; Karakiri et al., 1989).

In Dover sole (*Microstomus pacificus*), a commercially important northeast Pacific Ocean flatfish, metamorphosis differs from the rapid process described above. Markle et al. (1992) define five stages in Dover sole development. Stage 1 includes planktonic premetamorphic larvae from 6.1 to 58.5 mm standard length (SL). Eye migration begins when Stage-1 larvae are 10–15 mm SL but is arrested about midway through the process (Pearcy et al., 1977; Markle et al., 1992). Thus, Dover sole are optically asymmetrical during most of their planktonic life. Stage 2 includes planktonic metamorphic larvae from 42.3 to 60.4 mm SL, in which the eye has migrated past the mid-dorsal ridge and a pronounced shrinkage in body depth has begun. Stage 3 includes transitional metamorphic larvae from 40.7 to 74.9 mm SL, which are found in both the plankton and benthos. Stage-3 larvae are characterized by attainment of the adult configuration for a number of morphological characters. Stage 4 includes predominantly benthic, metamorphic larvae from 41.7 to 79.3 mm SL, which have formed a characteristic intestinal loop in the secondary body cavity. Stage 5 includes post-metamorphic juveniles from 48.9 mm SL to sexual maturity, which occurs at lengths greater than approximately 250 mm total length (Yoklavich and Pikitch, 1989; Hunter et al., 1992). Based on seasonal collections of staged larvae, Markle et al. (1992) estimated that duration of the metamorphic period (Stages 2, 3, and 4) is approximately 9 months.

The protracted metamorphic period in Dover sole provides an opportunity to examine changes in otolith structure and chemistry associated with each stage of metamorphosis. It may also be possible to distinguish between otolith landmarks associated with developmental processes and those associated with settlement from the water column to the bottom, because these events are not as coincidental in Dover sole as in other flatfish species. The objectives of this study were to 1) describe microstructure and microchemistry of Dover sole otoliths collected before, during, and after metamorphosis; 2) identify structural and chemical landmarks representing otolith growth during important morphological and behavioral transitions; 3) validate periodicity of increment formation in otoliths of metamorphic Stage-3 and Stage-4 larvae and post-metamorphic Stage-5 juveniles; and 4) using the otolith landmarks determined in objective 2 and increment counts between those landmarks and the otolith edge, re-examine the chronology of metamorphic events described in Markle et al. (1992).

Methods

Dover sole collections

Otoliths of six Stage-1, 103 Stage-3, 82 Stage-4, and 220 Stage-5 Dover sole were examined for microstructure and microchemical analysis. Specimens were obtained on various dates between 1987 and 1990 from bottom trawl collections off Oregon (Markle et al., 1992), opportunistic bottom trawl collections of commercial fishermen from Oregon and Washington, and midwater trawl collections off Oregon and central California[1] (Appendix 1).

The following measurements were taken to the nearest 0.1 mm on all specimens: standard length, body depth at anus (BD1A), and distance from snout to posterior extent of intestine (SINT). Weight of pat-dried specimens was determined to the nearest 0.1 g.

Otolith preparation and analysis for microstructure

Sagittae were removed after the fish were measured. Otoliths not immediately prepared for analysis were stored dry in vials. Terminology of otolith morphology follows Campana and Neilson (1985) and Secor et al. (1991), modified to account for features in Dover sole otoliths (Fig. 1 and Results section below). Left and right otoliths of Stage 3–5 Dover sole differ noticeably in shape (Fig. 1 and Results section below) and were analyzed separately.

The longest and shortest axes of sagittae from Stage-1 larvae were measured to the nearest 0.01 mm prior to mounting on slides in either a toluene-based medium (Histoclad) or an acrylic glue (Super Glue). Otoliths were then ground in the sagittal plane with 600-grit paper until growth increments became apparent.

Sagittae from Stage 3–5 Dover sole were cleaned in ethanol, air-dried, weighed to the nearest 0.01 mg, and measured along the anterior-posterior axis (maximum length) and along the dorsal-ventral axis (maximum width) to the nearest 0.01 mm (Table 1). The number of translucent rings (annuli) outside the clear central growth area (Fig. 1 and Results section below) was determined upon examination of whole otoliths under reflected light against a black background. Most otoliths were mounted on slides in a toluene-based medium (Histoclad) and the lateral face was ground in the

[1]Whipple, J. 1991. Progress in rockfish recruitment studies. U.S. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Southwest Fish. Sci. Center, P.O. Box 271, La Jolla, CA 92038. Admin. Rep. T-91-01, 57 p.

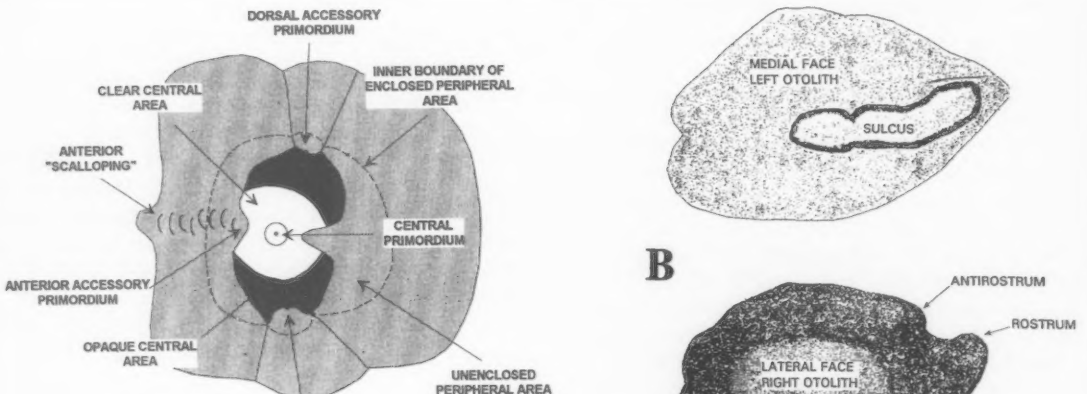

Figure 1

Schematic illustrations of Dover sole, *Microstomus pacificus*, otolith structure and orientation, showing terminology used in text. (**A**) Stage-4 left sagitta, sagittal section, lateral face towards the viewer, anterior to left. The anterior cavity is confluent with the anterior accessory primordium. (**B**) Orientation of left and right Stage-4 sagittae, anterior to right.

Table 1

Number of otoliths from Stage 3–5 benthic Dover sole, *Microstomus pacificus*, examined in study for each measurement or enumeration. AP = accessory primordium.

	Stage 3	Stage 4	Stage 5	Total
Right otoliths				
Whole otoliths				
Otolith length	81	53	215	349
Otolith width	81	53	215	349
Otolith weight	87	63	218	368
Number of annuli	87	63	209	359
Sagittal sections				
Diameter of clear central area	55	33	0	88
Number of AP	70	41	6	117
Increments since last AP (enclosed)	44	32	3	79
Increments since last AP (not enc.)	13	0	0	13
Increments since enclosure	51	46	3	100
Increments between checks	28	18	0	46
Left and right paired otolith comparisons				
Whole Otoliths				
Otolith length	67	50	198	315
Otolith width	67	50	196	313
Otolith weight	74	57	204	335
Sagittal sections				
Number of AP	41	22	0	63
Increments since last AP	23	12	0	35
First vs. last countable AP	14	11	0	25
Increments since enclosure	10	6	0	16
length anterior and posterior to AP	54	39	0	93

sagittal plane to the level of the central primordium. If resolution was not sufficient, the medial face was also ground to the central primordium. Otoliths from Dover sole >75 mm were ground and read progressively because all increments could not be observed in a single plane in sagittal section.

In order to examine otolith morphology in other planes, a subsample of otoliths from 17 Stage 3–5 Dover sole were embedded in low viscosity Spurr blocks (Haake et al., 1982) and ground to a level even with the central primordium in frontal or transverse sections. Ground sections were covered with a mounting medium and cover slip.

Additional otolith measurements were obtained from subsamples of Stage-3 and Stage-4 larvae (Table 1) to determine growth and development of features associated with metamorphic stages. The diameter of the clear central growth area (Fig. 1 and Results section below) of 88 right otoliths was measured to the nearest 0.02 mm on a black background at 50× with a dissecting scope and reflected light. Otoliths were then examined at 100, 400, and 1000× with a compound microscope and video monitor. At 100× we measured length of the otolith anterior and posterior to the central primordium to the nearest 0.01 mm in paired left and right otoliths from 93 larvae.

The number and position of accessory primordia, whether or not central primordium growth was completely enclosed by growth from accessory primordia, number of increments from accessory primordia to otolith edge, and number of increments from enclosure of central primordium growth to otolith edge were determined for all Stage-3 and Stage-4 larval otoliths in which the features were legible (Table 1). Similar information was obtained from 6 Stage-5 juveniles. Most counts and determinations were based on right otoliths; however, a subsample of left otoliths was also examined for paired comparisons (Table 1).

Counts of all growth increments from the first countable increment outside the central primordium to the otolith edge were made for five of six Stage-1 larval otoliths and for four Stage-3 larval otoliths exhibiting a variety of patterns of accessory primordium formation. Counts near the central primordium were made with 1000× light microscopy.

In all otoliths for which total increment counts were made and in approximately 20% of otoliths for which counts from accessory primordia were made, there were areas where accurate increment counts were not possible. In these areas, the number of increments was interpolated by linear approximation (Methot, 1983; Butler, 1989), based on the average widths of 5–20 increments on the distal or, preferably, both the proximal and distal sides of the uncountable area. Stage 3–5 larval otoliths with >5% interpolated increments were excluded from further analysis. We present the percentage of interpolated increments with total increment estimates for the few Stage-1 specimens examined.

Table 2
Rearing conditions of juvenile Dover sole, *Microstomus pacificus*, during the first validation experiment.

	Group 1	Group 2	Group 3
Collection depth	110 m	150 m and 163 m	108 m
Number injected	4	10	21
Number controls	13	0	0
Average weight[1]	5.4 g	3.4 g	4.5 g
Aquarium volume	152 L	76 L	114 L
Average density	0.036 g/L	0.045 g/L	0.039 g/L
Temperature	8°C	10°C	12°C

[1]Average for entire experiment, based on weights at death.

Validation of increment deposition rate

Two groups of juvenile Dover sole were given interperitoneal injections with a solution of 0.01 g oxytetracycline hydrochloride (OTC)/mL distilled water at a dose of 0.1 g OTC/kg fish weight (Campana and Neilson, 1982) to produce fluorescent marks on their otoliths. Dover sole in the first experimental group were held at three constant temperatures to evaluate temperature effects on increment formation. Those in the second experimental group were exposed to identical temperatures and were injected once in March and again in September to evaluate seasonal effects on increment formation.

First experiment An initial group of 48 juvenile Dover sole was collected at four stations off Cape Foulweather, Oregon, on 17 March 1990 (Table 2). Thirty-five fish were injected on that day and 13 served as uninjected controls to check for naturally occurring fluorescence. The fish were held in aquaria containing artificial seawater (Instant Ocean) and fed to satiation with *Tubifex* worms once per day in the morning. Fluorescent lights in the room were set to natural cycles, and aquaria were partially covered to reduce incoming light. Rearing conditions are summarized in Table 2.

Fish were sacrificed 21 (n=6), 26 (n=4) or 29 (n=9) days following initial injection. The remaining 29 fish were given a second injection of OTC 36 days after capture and were sacrificed 5 (n=6), 8 (n=10), or 12 (n=13) days after the second injection (i.e., 41, 44, or 48 days after initial injection).

Second experiment A second group of 10 juvenile Dover sole ranging from 55.3 to 118.3 mm was collected on 20 March 1991 at 77 m off Cape Foulweather, Oregon, and injected the following day. The fish were held in a flow-through filtered seawater tank. Ambient nearshore oceanic water entering the tank averaged 10.7°C (range: 8.7–14.6°C) during the experiment. Fish were exposed to diffused natural light and fed as in the first experiment. Seven days after injection, four fish, 55.3–63.9 mm, were sacrificed. The remaining six fish were individually marked with fin clips on 27 April. They were re-injected on 11 September, 174 days after first injection, and sacrificed 19 days later. Lengths ranged from 88.2 to 152.2 mm.

Otoliths from both groups of fish were prepared as described above and examined at 100–1000× on a Zeiss microscope equipped with a IV Fl epifluorescence condenser. The preparation was examined under full spectrum light to locate areas with distinct growth increments and the OTC band was then observed under ultraviolet light.

At least two documentary 35-mm slides of each otolith were taken: one showing the fluorescent band as well as the otolith edge and one showing increments under full-spectrum illumination. Each slide was projected and traced onto the same sheet of paper, resulting in a diagram that showed the location of the fluorescent band in relation to increments and other landmarks. The diagram served as documentation for increment counts as well as a guide for further examination with light microscopy. Photographs and counts were made at one to four locations on each otolith, usually at the rostrum, antirostrum, or post-rostrum (Fig. 1). Increment counts were generally made on right otoliths; left otoliths were used only if right otoliths were unavailable or illegible. All otoliths were read at least twice on separate dates by the same reader and the average was used in subsequent analyses. The range of estimates for each otolith and standard deviation of mean counts were also determined.

Results were analyzed by regressing number of increments between the proximal edge of the innermost fluorescent band and edge of the otolith against number of days since OTC injection. When a second fluorescent band was obvious and the area distal to the second band was illegible, counts were made between bands. To determine if all experimental groups could be combined into one regression, multiple regressions, including "dummy variables" corresponding to experimental groups, were analyzed with partial F-tests (Neter et al., 1989:364–370). The slope of the final regression model was compared to a slope of 1.0 (Neter et al., 1989).

Otolith preparation and analysis for microchemistry

Otoliths from two Stage-3 larvae (51.3 and 61.9 mm), three Stage-4 larvae (54.6, 57.1, and 65.7 mm), and two Stage-5 juvenile Dover sole (88.6 and 172.2 mm) were examined with a wavelength dispersive electron microprobe to determine if microchemical changes were associated with microstructural changes. Preparation and analytical techniques followed Toole and Nielsen (1992). Beam diameter was 5 µm, counting time for each element was 20 seconds, accelerating voltage was 15 kV, and current was 20 nA.

Results

Validation of increment deposition rate

First experiment Otoliths from uninjected control fish exhibited bright yellow-green fluorescence around the edge of the otolith and weaker fluorescence associated with strong checks and scratches. However, fluorescent marks observed on injected fish differed markedly in appearance. Twenty-seven injected fish had at least one otolith with one or two distinct fluorescent bands located inside the otolith edge (Fig. 2, A and B). Fluorescent bands extended over two to six increments.

Increments formed after capture were often less distinct than those formed in nature (Fig. 3), and four of 27 injected fish with fluorescent bands (14.8%) had otoliths with such poorly defined increments that they were considered unreadable and were eliminated from further analysis.

Both daily and subdaily increments (Campana and Neilson, 1985) were observed. For those otoliths in which both subdaily and larger increments could be counted in the same area, the ratio of small to large increments averaged 2.78 (n=10 fish, range 1.36–3.48). In most cases, larger growth increments could easily be distinguished from subdaily increments by adjusting the focus. However, in otoliths of two fish (7.4% of those with fluorescent bands), only subdaily increments could be observed. These fish were also eliminated from further analysis.

Second experiment Four Dover sole sacrificed 7 days after first injection had not fed. The remaining six fish began feeding approximately two weeks after the first OTC injection. Two of these fish grew rapidly throughout the experiment, whereas the other four fish ceased growing after about 20 weeks (Fig. 4).

Two of four Dover sole sacrificed 7 days after first injection and five of six fish sacrificed 19 days after second injection had at least one otolith with a

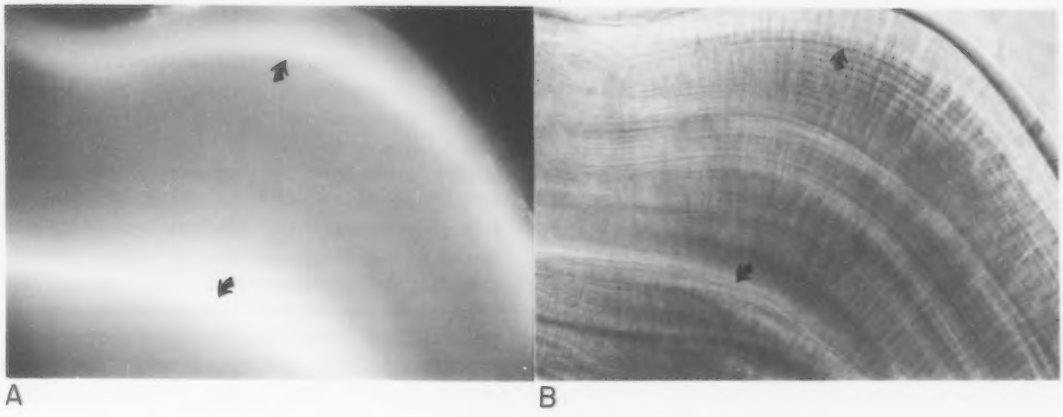

Figure 2

Sagittal section of right otolith from 67.2 mm Stage-4 Dover sole, *Microstomus pacificus*, larva marked with OTC and held in a 12°C aquarium for 48 days. Magnification = 400×. (**A**) Photograph taken with ultraviolet light; arrows show fluorescent OTC marks. (**B**) Same preparation taken with full-spectrum light; arrows show position of fluorescent OTC marks.

fluorescent mark and countable increments (70% of injected fish). Increment width was narrow, ranging from 0.63 to 1.88 µm. Because all counts underestimated the true number of days since injection and because larger marks were not apparent by adjusting focus, the marks were not equivalent to subdaily marks observed in the first experiment. The OTC mark deposited after first injection was not visible in fish held until second injection, so increments deposited during the entire 183-day period could not be counted.

Precision of counts Maximum and minimum increment counts for each otolith differed from mean counts by an average of 5.7% (Range=0–20%). Standard deviations associated with mean increment counts averaged 1.84 (Range=0–7.8).

Validation relationships Neither variances (Bartlett's test, B=1.48, P=0.11) nor mean observed/expected increment counts (ANOVA, df=27, F=2.21, P=0.10) differed between the five experimental groups of fish (three aquarium temperatures in the first experiment, March and September marking groups in the second experiment). Similarly, regressions of observed versus expected counts did not improve when a model containing separate slopes and intercepts for each experimental group was compared with a model containing separate intercepts and one slope (F=0.313, df=5,22,

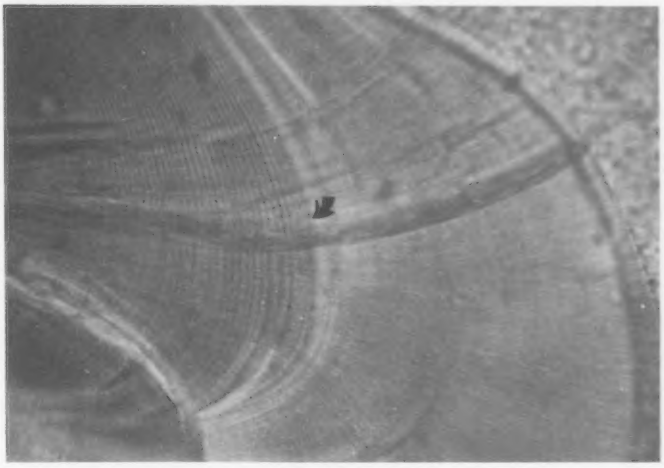

Figure 3

Left otolith, anterior end of frontal section; from 68.0mm Stage-4 Dover sole, *Microstomus pacificus*, larva marked with OTC and held in a 10°C aquarium for 44 days. Arrow marks capture point, as identified by OTC fluorescence. Increments prior to capture are well-defined, whereas increments formed in aquarium are nearly indiscernible. Magnification = 400×.

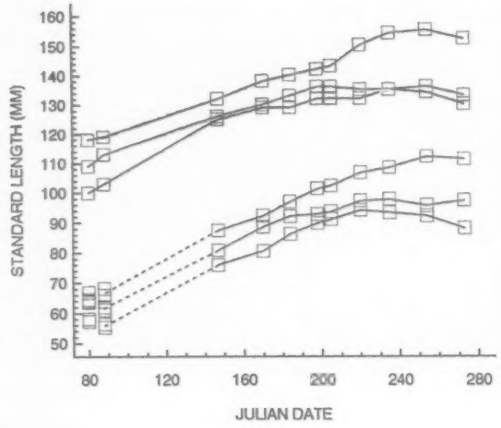

Figure 4

Relationship between standard length and calendar date of Dover sole, *Microstomus pacificus*, held in an ambient flow-through seawater tank, 21 March–30 September 1991 (Julian dates 80–272). Smaller fish were not marked initially; dotted lines indicate uncertainty regarding growth trajectories.

P=0.899) or when the model with separate intercepts and one slope was compared with a model containing one slope and one intercept (F=1.14, df=5,22, P=0.369), so all experimental groups were initially combined into one regression. However, inspection of residuals from that regression suggested that a greater error was associated with counts from Stage-5 juveniles than with counts from Stage-3 and Stage-4 larvae, regardless of experimental group. Mean ratios of observed:expected counts for fish <80 mm (0.924) than for fish ≥80 mm (0.718) (t-test, df=26, P=0.012). Therefore, each size group was treated separately in the final regression models.

The relationship for Dover sole <80 mm was

Days since marking = 3.81 + (0.962 * observed increments),

where n=21, r^2 = 0.85, $SE_{INTERCEPT}$ = 2.90, and SE_{SLOPE} = 0.091 (Fig. 5A). The calculated slope was not significantly different from 1.0 (t^*=0.419, df=26, P=0.34); however, the power of this test (Peterman, 1990) was too weak to conclude that increments were deposited daily (1–ß≈0.06; Rice, 1987, Neter et al., 1989).

The relationship for Dover sole ≥80 mm was

Days since marking = 6.32 + (1.026 * observed increments)

where n=7, r^2 = 0.93, $SE_{INTERCEPT}$ = 2.81, and SE_{SLOPE} = 0.122 (Fig. 5B). The calculated slope was not significantly different from 1.0 (t^*=0.211, df=6, P=0.42); however, the power of this test was also too weak to conclude that increments were deposited daily (1–ß≈0.05).

Error in the preceding regressions was due, in part, to poor resolution of laboratory-formed increments (Fig. 3; Campana and Neilson, 1985) and may therefore be greater than the error associated with ageing wild-captured fish. However, several resolution problems appeared common to otoliths of wild-captured and laboratory-reared fish: difficulty interpreting increments at the otolith margin, difficulty resolving increments around stress checks (Campana and Neilson, 1985) in certain regions of the otolith because of compression (and sometimes fusion) of increments, and occasional difficulty distinguishing daily and subdaily increments. For these reasons, use of both slope and intercept estimates was considered reasonable when back-calculating days from increments in otoliths of wild-captured fish. The same relationships were applied to increment counts outside the range of observations, which may introduce an unavoidable source of additional error.

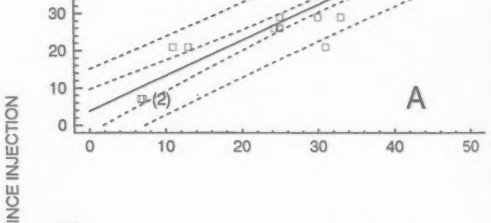

Figure 5

Relationship between days since OTC injection and number of growth increments in Dover sole, *Microstomus pacificus*, otoliths. Inner dashed lines are 95% confidence limits for mean response; outer dashed lines are 95% prediction limits. (**A**) Relationship for Stages 3 and 4 <80 mm SL. Formula: days since injection = 3.81 + (0.96 * observed increments); n=21; r^2=0.85. (**B**) Relationship for Stage-5 Dover sole ≥80 mm SL. Formula: days since injection = 6.32 + (1.03 * observed increments); n=7; r^2=0.93.

Table 3

Characteristics of five Stage-1 Dover sole, *Microstomus pacificus*, otoliths. Number of increments equals number observed plus number interpolated by linear approximation. The range of values presented for one larva reflects uncertainty in interpreting possible subdaily increments.

Standard length (mm)	Collection date	Maximum diameter (mm)	Number of increments	Percent interpolated (%)	Mean increment width (μm)
30.1	January, 1987	0.10	77	52	1.30
20.6	March, 1991	0.15	103	33	1.46
46.0	July, 1991	0.25	160–180	0	1.39–1.56
51.5	July, 1991	0.28	229	20	1.22
47.7	Oct., 1991	0.30	182	5	1.64

Structural patterns

Growth from central primordium Two prominent areas were seen within the field of growth emanating from the central primordium.

Clear central area Stage-1 larval otoliths were translucent and hemispheric (appearing nearly circular in sagittal section), with all growth emanating from the central primordium (Figs. 1 and 6). The left eye had previously migrated to the dorsal ridge of the cranium in all Stage-1 larvae examined. The diameter of Stage-1 larval otoliths ranged from 0.10 to 0.30 mm and the number of increments ranged from 77 to 229 (Table 3). When fully formed in Stage-3 larval otoliths, this clear central area was 0.24–0.49 mm (mean=0.39, n=60) and contained 187–230 increments (Table 4). Mean increment width in the clear central growth area ranged from 1.22 to 1.56 μm, and increment widths nearer the central primordium were approximately 0.5 μm, suggesting that, when counting increments, underestimation may have occurred owing to resolution limitations of light microscopy (Campana et al., 1987).

A structural feature of some Stage-1 larval otoliths was the presence of one or two conical cavities, which radiated towards the anterior and posterior edges (Fig. 7). Each cavity was enclosed laterally and medially and was open at the anterior or posterior end. The anterior cavity may be continuous with the sulcus, which was visible on one Stage-1 larval otolith.

Opaque central area An opaque area surrounded and was continuous with the clear central area in all Stage 3–5 larval otoliths (Figs. 1 and 8, A–D). Increments in this region were wider than increments in the clear central area (1.6–1.8 μm) and there was higher contrast between continuous and discontinuous zones within increments. Continuity of increments was interrupted by the conical cavities described previously and by accessory primordia. The number of increments in this area ranged from 46 to 95 (Table 4).

Growth from accessory primordia We recognized two zones of growth from accessory primordia, distinguished by the point at which growth from the central primor-

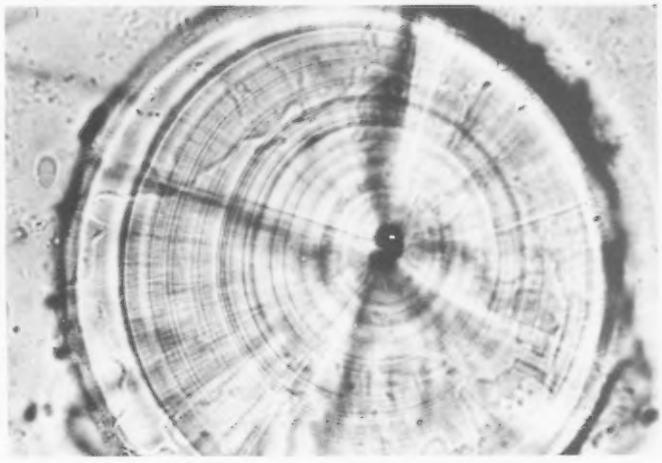

Figure 6

Sagitta from 20.6 mm Stage-1 Dover sole, *Microstomus pacificus*, larva collected on 1 April 1990 at NMFS Station 9003-35. Estimated number of increments was 103 (Table 3). Magnification = 1000×.

Table 4

Increments counted from central primordium to structural landmarks on four Stage-3 Dover sole, *Microstomus pacificus*, otoliths shown in Figure 8, A–D. Assigned dates (in parentheses) are determined from the increment:day regression for growth distal to accessory primordia. Periodicity of increments proximal to accessory primordia has not been validated. Ranges indicate uncertainty interpreting possible subdaily increments or determining exact location of the clear to opaque transition. The first distinct accessory primordium is not the first-formed accessory primordium. NP = not present; AP = accessory primordium.

	Standard length of larvae (mm)			
Landmark	55.6	46.9	64.9	55.5
Central primordium	0	0	0	0
Clear to opaque transition	187–213	205–219	≈230	≈200
First distinct AP	196–222 (19 Nov. 88)	NP	322 (3 Dec. 89)	206 (17 Dec. 89)
Last AP	224–250 (16 Dec. 88)	NP	338 (18 Dec. 89)	222 (1 Jan. 90)
Central primordium enclosure	NP	300–314 (20 Feb. 90)	376 (24 Jan. 90)	252 (30 Jan. 90)
Otolith edge	255–281	322–336	425	296
Capture date	19 Jan. 89	17 Mar. 90	17 Mar. 90	17 Mar. 90

dium was completely enclosed. This determination is based on sagittal sections at the level of the central primordium.

Unenclosed peripheral area Otoliths of all Stage 3–5 larvae had at least two accessory primordia (AP), when viewed in sagittal section (Fig. 8, A–D). A maximum of seven AP were observed, although additional AP on the lateral surface of the otolith (Fig. 9) were obscured when otoliths were prepared in sagittal section. Accessory primordia always formed adjacent to growth from the central primordium, rather than adjacent to growth from previously formed AP. On right otoliths, AP were easier to discern than on left otoliths, possibly because the plane of growth changes at about the time of AP formation in left, but not right, otoliths (Fig. 10, A and B). Increments emanating from AP exhibited higher contrast and were wider (about 3.0 µm) than those emanating from the central primordium.

The most anterior AP appeared to form first and was closely associated with the transition from clear to opaque central areas. However, the exact origin of the anterior AP was usually impossible to discern because it merged

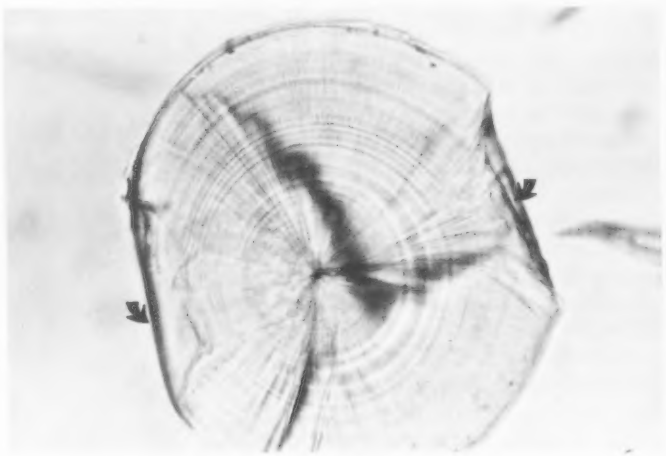

Figure 7

Sagitta from 51.5 mm Stage-1 Dover sole, *Microstomus pacificus*, larva collected on 12 July 1991 near Destruction Island, Washington. Anterior and posterior cavities are indicated by arrows. The lateral face of the otolith (surface toward viewer) has been ground to the level of the central primordium; the medial face has been left intact. Estimated number of increments was 229 (Table 3). Magnification = 400×.

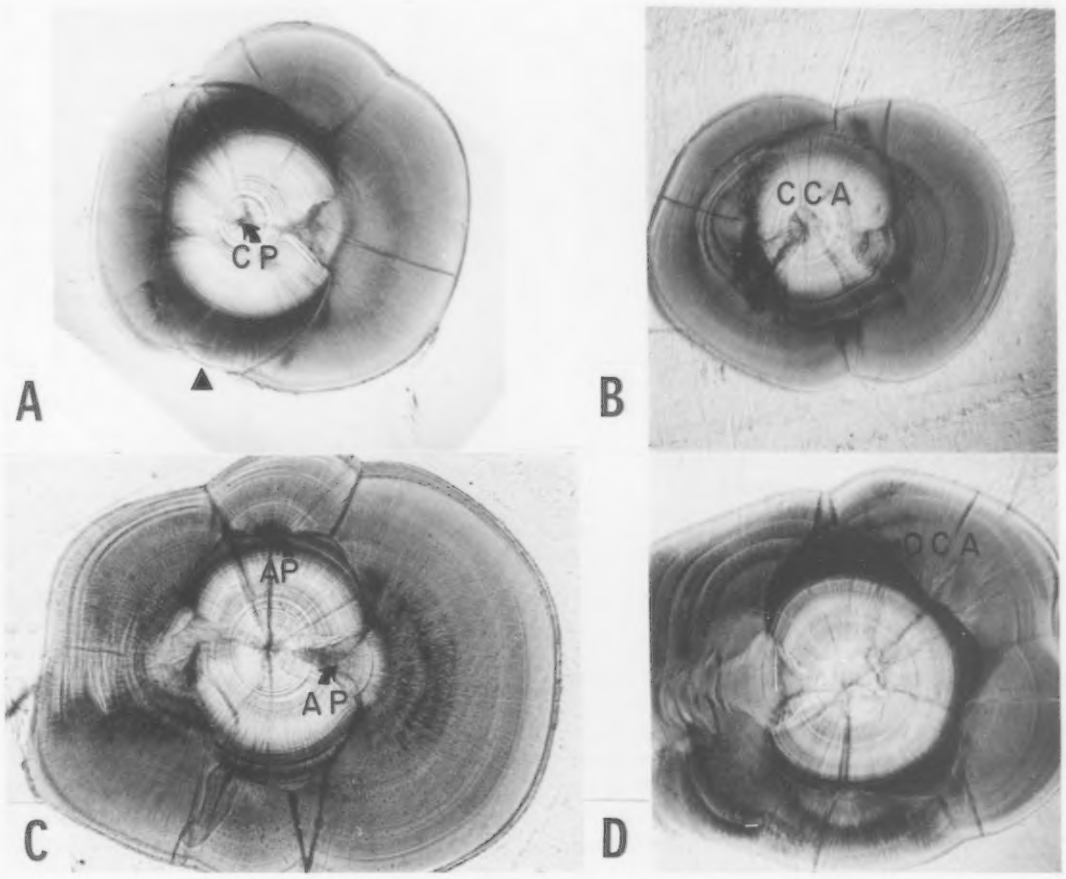

Figure 8

Stage-3 Dover sole, *Microstomus pacificus*, otoliths showing variations in accessory primordium formation; anterior to left, dorsal toward top in A–C, dorsal toward bottom in D. AP = accessory primordium, CCA = clear central area, CP = central primordium, OCA = opaque central area. (**A**) Left otolith of 55.6-mm larva collected 19 January 1989, showing unenclosed central primordium growth (indicated by triangle). Magnification = 100×. (**B**) Left otolith of 46.9-mm larva collected on 17 March 1990, showing two accessory primordia (anterior and posterior) completely enclosing growth from the central primordium. Magnification = 100×. (**C**) Left otolith of 64.9-mm larva collected 17 March 1990, showing growth from four accessory primordia (anterior, posterior, dorsal, and ventral) completely enclosing growth from the central primordium. Magnification = 100×. (**D**) Right otolith of 55.5-mm larva collected on 17 March 1990, showing growth from four accessory primordia (one anterior, two posterior, and one dorsal) completely enclosing growth from the central primordium. Magnification = 100×.

with the conical cavity described previously (Fig 8, A–D). This situation also applied to the origin of the most posterior AP in left otoliths (Fig. 8, A–C). The chronology of additional AP formation did not follow a consistent pattern. Dorsal and ventral AP were often associated with dark bands in the opaque central area (Fig.11).

Dorsal and ventral AP were formed 196–338 increments distal to the central primordium (Table 4). When increments were counted back from the otolith edge, dates of last AP formation varied between fish, ranging over a six-month period from October to March (Fig. 12). The number of days between the first-formed and last-formed dorsal and ventral AP averaged 28.8 (range 1–75, n=25). This range would be greater if it were possible to make accurate counts to the anterior AP.

Enclosed peripheral area Growth from accessory primordia completely enclosed growth from the cen-

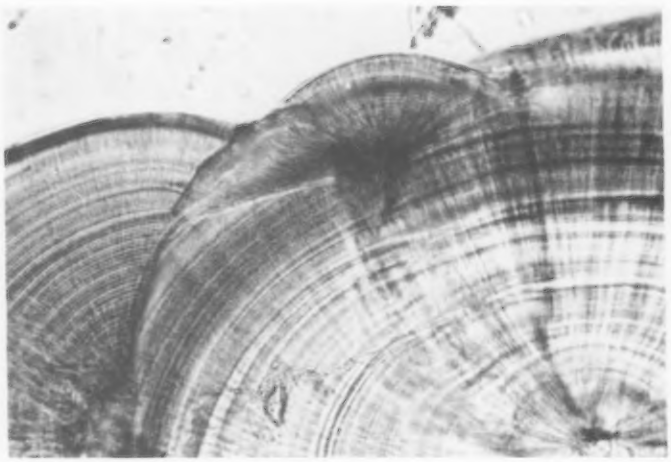

Figure 9
Transverse section of right otolith of 47.5-mm Stage-3 Dover sole, *Microstomus pacificus*, larva collected on 22 March 1989, showing recently-formed accessory primordium near medial surface (toward top) that would be undetectable in sagittal section. Magnification = 400×.

tral primordium in at least one otolith of 78% of benthic and 71.4% of pelagic Stage-3 larvae, 98.4% of benthic and 100% of pelagic Stage-4 larvae, and 100% of Stage-5 larvae, suggesting that most otoliths were enclosed before or during Stage 3. Enclosure of growth from the central primordium occurred an average of 33.7 days after formation of the last accessory primordium (range 0–87, n=108).

During Stages 3 and 4, SINT increases as the intestinal loop extends into the secondary body cavity and BD1A decreases as body depth shrinks (Markle et al., 1992). Consequently, fish with low SINT/BD1A ratios are presumably at an earlier stage in the metamorphic process than fish with higher ratios. Benthic Stage-3 larvae with at least one unenclosed otolith had a lower ratio of SINT/BD1A (mean=1.06) than benthic Stage-3 larvae with enclosed otoliths (mean=1.15) (t-test, df=46, P=0.0096), as did pelagic Stage-3 larvae (0.90 vs. 1.32, t-test, df=28, P=0.019), suggesting that Stage-3 larvae with unenclosed otoliths were at an earlier stage of development than Stage-3 larvae with enclosed otoliths.

Stage-3 larvae with enclosed otoliths were collected an average of 24.4 days after enclosure (range= 5.7–65.4, n=51) while Stage-4 larvae in the same collections averaged 51.9 days (range=9.6–125.9, n=46). The difference (27.5 days) may represent the average duration of Stage 3.

Otolith elongation along the anterior-posterior axis became more pronounced following enclosure, as did asymmetry between left and right otoliths. Left otoliths were heavier (paired t-test, df=335, P<0.001), longer (paired t-test, df=314, P<0.001) and wider (paired t-test, df=312, P<0.001) than right otoliths. In addition, the proportion of otolith length anterior to the central primordium was higher in right than left otoliths (t-test, df=92, P<0.001) by an average of 8.5% (Fig. 8, A–D). The number of accessory primordia tended to be higher on right than on left otoliths (paired t-test, df=62, P=0.036), while more increments formed after the last AP (paired t-test, df=34, P=0.006) and after the point of enclosure (paired t-test, df=15, P=0.011) on left than on right otoliths.

Stress checks became prominent in sagittal section following enclosure (Fig. 8, B–D). Enumeration of stress checks and determination of periodicity was difficult because checks were often discontinuous around the otolith and, even when continuous, the number of observable increments between checks varied regionally. However, mean periodicity of check formation in 46 Stage-3 and Stage-4 otoliths collected over a three-day period in March, 1990, suggested synchrony of check formation in otoliths of different fish and regularity in periodicity of check formation (Fig. 13). Calculated dates of check formation did not correspond to particular lunar phases. Number of days between checks ranged from 5.7 to 33.6, with a mode of 12.5 days and an average of 15.3 days (Fig. 14).

Other features of the enclosed peripheral zone in older fish were the development of a rostrum (Figs. 1 and 15) and formation of translucent growth zones (Fig. 16). All Stage-5 otoliths collected in January and March had at least one translucent growth zone. This first post-settlement translucent growth zone was initiated in late fall of the settlement year (240–293 days after formation of the last AP) and was completed the following spring (125 to 141 days later) in otoliths of three Stage-5 juveniles. Based on daily increment counts and seasonal deposition patterns, the first and subsequent translucent growth zones are interpreted as post-settlement annuli formed during slow winter growth periods (Fig. 17, page 746). This interpretation is consistent with Hagerman (1952) and Chilton and Beamish (1982).

Although there was little difference in weight between the largest Stage-4 and smallest Stage-5 Do-

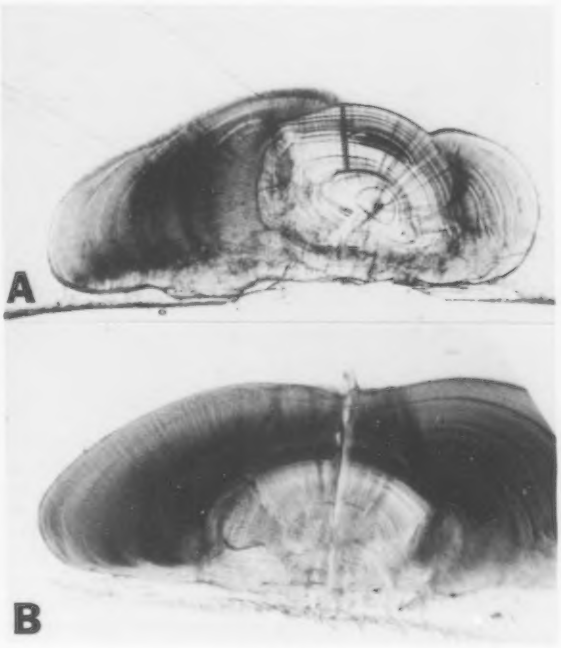

Figure 10

Frontal sections of left and right Dover sole, *Microstomus pacificus*, otoliths showing differences in orientation of the clear central growth area; anterior to right, lateral surface to top. (**A**) Left otolith from 58.0-mm Stage-3 larva collected on 16 March 1990. Central area tilts towards the lateral surface posteriorly and towards the medial surface anteriorly. (**B**) Right otolith from 64.9-mm Stage-3 larva collected on 17 March 1990. Central area is oriented in the same direction as surrounding peripheral growth.

ver sole collected in January and March, otolith weight in Stage-5 juveniles was nearly double that of Stage-4 larvae (Fig. 18, page 746). A discontinuity in the relationship between Stage 3–5 right otolith length and fish length during the first winter following settlement was also apparent (Fig. 19, page 746). This relationship was best described by a three-stanza segmented linear model (Bacon and Watts, 1971; Laidig et al., 1991). Parameter estimates in Table 5 (page 746) resulted in a good fit (r^2=0.955) and no discernable pattern in residuals. The first segment consisted of Stage-3 and Stage-4 larvae; the second, of Stage-4 larvae and Stage-5 juveniles; and the third, of only Stage-5 juveniles.

Microchemical patterns

Ratios of Sr/Ca exhibited similar patterns along transects through the otoliths of all larvae and juveniles examined (Figs. 20, A–B, page 747, and 21, page 748). An initial Sr/Ca spike (0.007–0.010) occurred approximately 48 µm from the central primordium, inside the clear central area. Following the spike, Sr/Ca levels within the clear central area fluctuated at intermediate levels (0.004–0.006). Sr/Ca ratios began a second, more gradual, decline beginning near the clear-to-opaque transition in the central area of the otolith, which also corresponded to the area in which the anterior AP was forming. This decline began in late summer or fall of the calendar year prior to settlement.

Sr/Ca ratios often reached minimum levels following formation of accessory primordia. In a 65.7-mm Stage-4 larval otolith (Fig. 20A), Sr/Ca ratios reached a minimum (0.002) at about the end of January. This minimum point was approximately 10 days beyond enclosure of growth from the central primordium and 31 days beyond the most recently formed accessory primordium. In a second Stage-4 larva, a minimum (0.003) was first reached in mid-De-

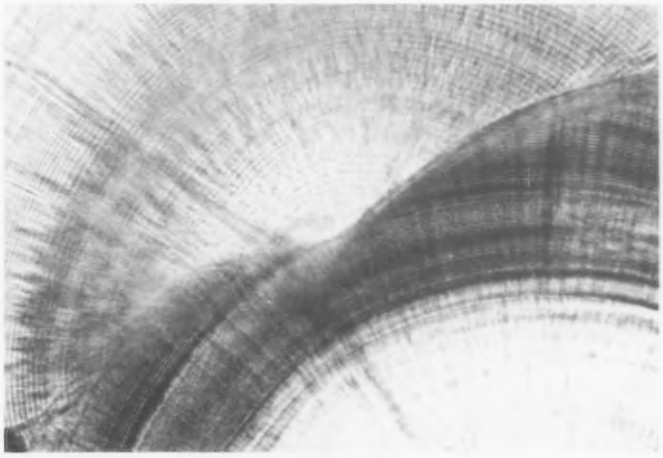

Figure 11

Posterior accessory primordium on right otolith of 58.0-mm Stage-3 Dover sole, *Microstomus pacificus*, larva collected on 16 March. Narrow dark band in the opaque central area is associated with origin of the accessory primordium. Magnification = 400×.

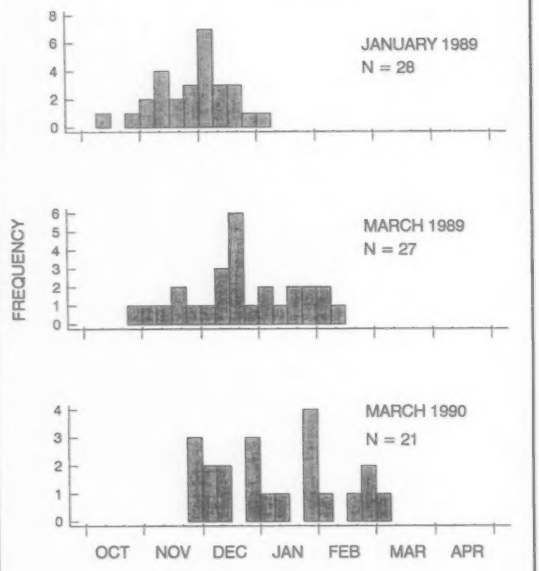

Figure 12

Dates of last accessory primordium formation on right otoliths of Stage-3 and Stage-4 Dover sole, *Microstomus pacificus*, larvae collected during three sampling cruises in 1989 and 1990. Only enclosed otoliths with three or more accessory primordia were considered because origins of single anterior and posterior accessory primordia are usually indistinct.

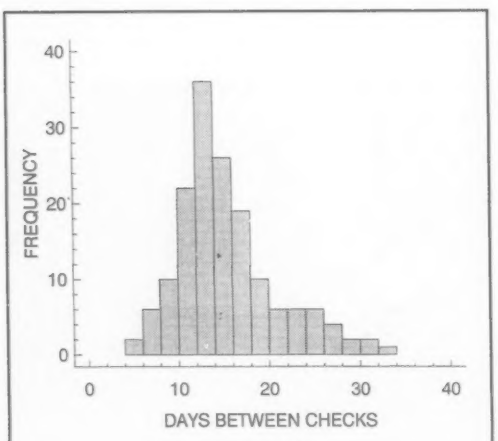

Figure 14

Frequency distribution of number of days between the five most distal stress checks on 46 Stage-3 and Stage-4 Dover sole, *Microstomus pacificus*, otoliths collected between 15–17 March 1990 (n=158).

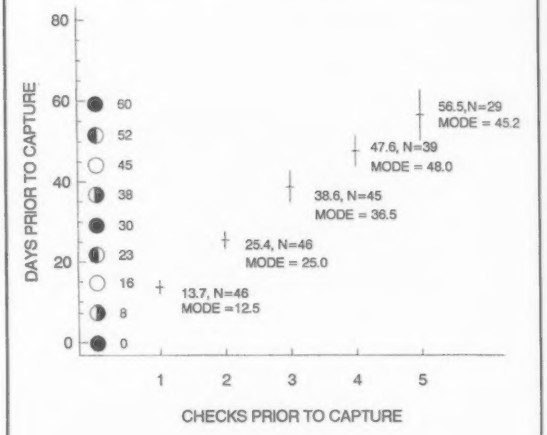

Figure 13

Relationship between the five most distal stress checks and number of days from their formation to the otolith edge (capture) in 46 Stage-3 and Stage-4 Dover sole, *Microstomus pacificus*, otoliths collected between 15–17 March 1990. Vertical bars represent 95% confidence intervals for means (horizontal bars). Additional error is associated with converting increments to days, with the regression described in the text. Corresponding lunar phases are displayed on the vertical axis.

cember. The ratio then rose and fell to a second minimum (0.003) in mid-January. In each of these larvae, the ratio rose again (0.003–0.0035) prior to capture in mid-March. Two Stage-3 larvae and a third Stage-4 larva did not exhibit distinct minima; rather, Sr/Ca ratios remained at about 0.0025 until capture. Sr/Ca ratios first reached this level prior to enclosure.

An 88.6-mm Stage-5 juvenile (Fig. 20B) with one peripheral annulus had narrow growth increments, making it difficult to determine exact ages associated with microprobe samples. However, the pattern proximal to AP was similar to that of larvae, with one 0.007 spike and a 0.004–0.006 peak near the center of the otolith, followed by a decline occurring prior to and during formation of accessory primordia. Sr/Ca ratios remained low for >200 days following formation of the last AP until a second 0.004–0.005 peak formed in the translucent annulus near the otolith edge, which represented capture in March.

A 172.2-mm juvenile, collected in November 1989 and judged to have three complete post-settlement annuli and a fourth one forming, had four Sr/Ca peaks, which corresponded to translucent annuli (Fig. 21), distal to accessory primordia.

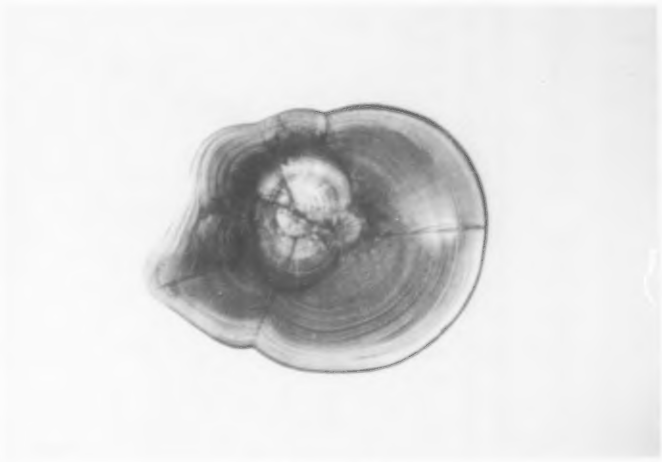

Figure 15
Left otolith of 65.0-mm Stage-4 Dover sole *Microstomus pacificus* larva collected on 17 March 1989; anterior to left. Note stress checks, which are clearest in the anterior field behind the anti-rostrum. Magnification = 50×.

Discussion

Relation of otolith microstructure to metamorphic stages

Premetamorphic larvae Sagittal otoliths of Stage-1 larvae between 20.6 and 51.5 mm were uniformly translucent and lacked accessory primordia. Since the left eye had migrated to the middorsal ridge of the cranium in each specimen, formation of AP is not triggered by the initiation of eye migration or movement to this position in Dover sole. Cavities noted in Stage-1 larval sagittae have not been described previously and their derivation and function are unknown.

Metamorphic larvae Because Stage-2 larval otoliths were not available, the transition from clear to opaque central areas and development of the first two accessory primordia cannot be attributed to this stage with certainty. However, because neither of these features were developed in Stage-1 larval otoliths, yet both were present in all Stage-3 larval otoliths, it is probable that they formed during Stage 2. Thus, the transition from clear to opaque growth and initiation of AP formation occur after the left eye moves beyond the dorsal ridge and metamorphosis (as defined in Markle et al., 1992) begins, but before larvae are first collected on the bottom.

Formation of the first accessory primordium in plaice and winter flounder also does not occur until the left eye has migrated at least to the dorsal ridge of the cranium (Alhossaini et al., 1989; Sogard, 1991). However, unlike Dover sole, AP in otoliths of these species can form before the eye has moved past that point. Comparisons with other flatfish species are more difficult. Accessory primordia form "at or shortly after metamorphosis" in starry flounder (Campana, 1984a) and "after metamorphosis" in California halibut (Kramer, 1991); however, the definition of metamorphosis relative

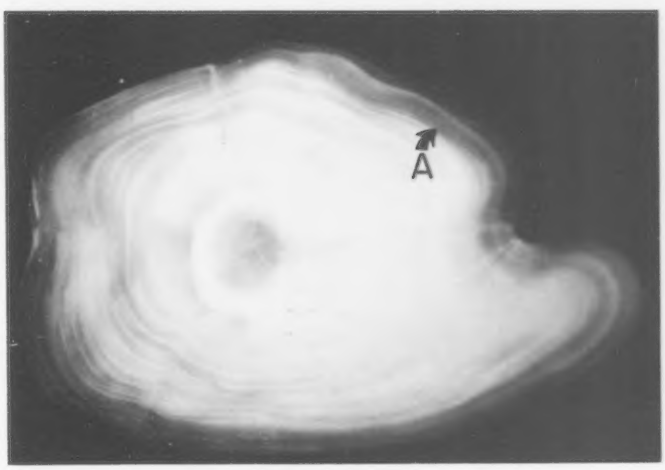

Figure 16
Right otolith of 100.4-mm Stage-5 juvenile Dover sole, *Microstomus pacificus*, caught on 18 March 1991 (anterior to right), photographed with reflected light against black background. Note the first annulus, which appears darker than the inner area. Magnification = 50×. A = annulus.

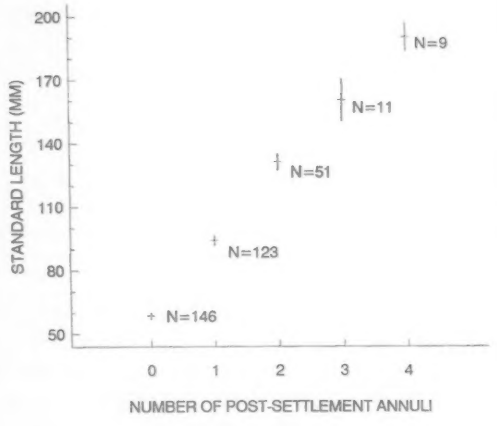

Figure 17

Relationship between standard length and number of post-settlement annuli in Dover sole, *Microstomus pacificus*, collected in January 1989, March 1989, and March 1990. Vertical bars represent 95% confidence intervals for means (horizontal bars).

to cranial morphology was not specified in these studies.

Formation of accessory primordia has also been noted in several non-pleuronectiform taxa (Brothers, 1984). Gartner (1991) described a clear to opaque transition with accompanying formation of AP in otoliths of myctophid species and suggested these features were

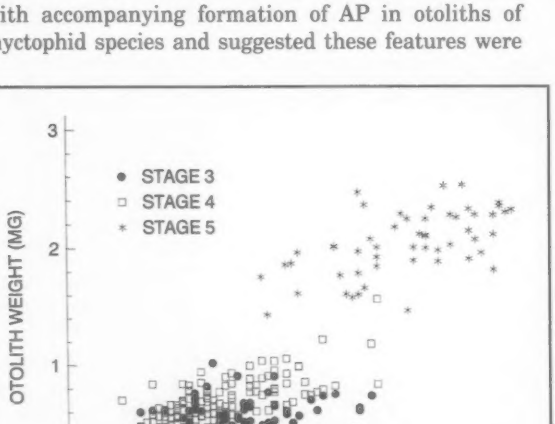

Figure 18

Relationship between weight of right otolith and body weight in Stages 3–5 Dover sole, *Microstomus pacificus*. Note change in the relationship during the transition from Stage 4 to Stage 5 (n=368).

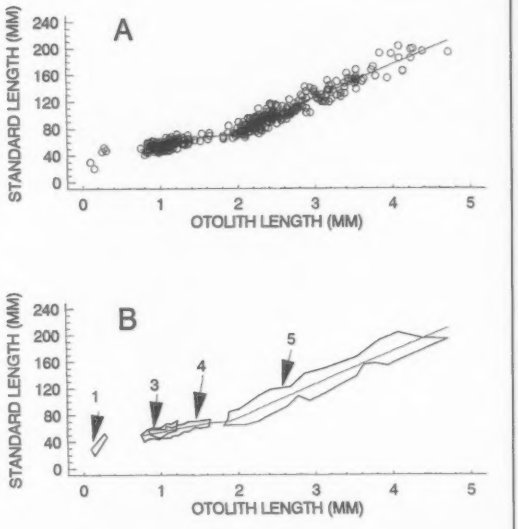

Figure 19

(**A**) Relationship between standard length and right otolith length in Stages 3–5 Dover sole, *Microstomus pacificus*. Formula and parameter estimates are included in Table 5. n = 351; r^2 = 0.955. Points representing standard length and right otolith diameter of five Stage-1 larvae are also included. While Stage-2 otoliths were not available, it appears that otolith length increased between Stage 1 and Stage 3, whereas fish length did not. (**B**) Polygons circumscribing areas bounded by fish in Stages 1 and 3–5.

Table 5

Estimated parameters for segmented linear regression model of the form

$$\text{Standard Length (mm)} = e + d_1(x-c_1) + d_2(x-c_1)s_1 + d_3(x-c_2)s_2,$$

where x = right otolith length (mm), s_i = [2*(1/(1+exp(−[x−c_i]/0.1)))]−1 (i.e., the logistic cumulative distribution function, used as a transition function between segments, with the constant 0.1 specifying a rapid transition between segments), c_i = fitted inflection points, and e and d_i = fitted parameters (n = 351, r^2 = 0.955).

Parameter	Estimate	Standard error
e	63.753	36.999
c_1	1.640	1.045
c_2	1.891	0.230
d_1	35.631	1.920
d_2	−6.485	40.950
d_3	19.999	41.233

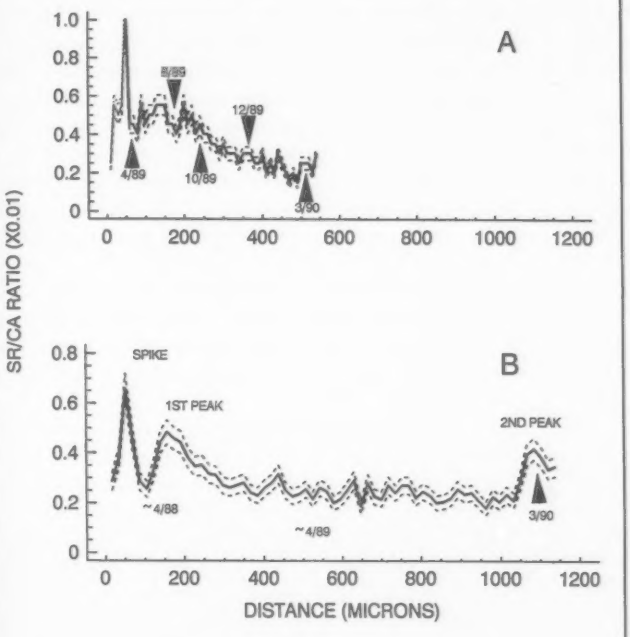

Figure 20

Relationship between Sr/Ca ratios and distance from central primordium towards edge in Dover sole, *Microstomus pacificus*, otoliths. Distances are not comparable between otoliths because transects were not oriented identically. Approximate dates are indicated, based on counts of increments between microprobe marks and the otolith edge. Dashed lines represent 2-σ measurement error, calculated as in Toole and Nielsen (1992). (**A**) 65.7-mm Stage-4 larva collected on 17 March 1990. (**B**) 88.6-mm Stage-5 juvenile collected on 17 March 1990. This fish had one annulus forming at the otolith edge, corresponding to the second Sr/Ca peak.

associated with a habitat transition from warm surface waters to colder midwater depths during the larval to juvenile transition. Dover sole could experience a similar temperature change at the time of AP formation, as discussed below.

Because growth from the central primordium was enclosed by growth from AP in otoliths of most Stage-3 larvae and because new AP were never observed distal to the point of enclosure, the last AP generally formed during either Stage 2 or Stage 3. The presence of very recently formed AP (<7 days) in some Stage-3 larvae and the possibility that additional AP could form in 22% (benthic) to 29% (pelagic) of unenclosed Stage-3 larval otoliths suggest that formation of the last accessory primordium may have occurred during Stage 3, rather than during Stage 2, in some individuals. Because Stage-3 larvae with unenclosed otoliths had lower SINT/BD1A ratios than Stage-3 larvae with enclosed otoliths, it appears that when the last AP does not form during Stage 2, it forms early in Stage 3. Thus, completion of AP formation may occur during the final stages of eye migration, when Dover sole larvae are still pelagic, or after completion of eye migration, shortly after Dover sole first settle to the bottom. In virtually all cases, it occurs before the intestinal loop begins to extend into the secondary body cavity.

Occasional formation of the last AP after settlement in Dover sole corresponds to observations of AP formation in other species. Alhossaini et al. (1989) described unenclosed otoliths of benthic plaice larvae, suggesting that AP formation may not be complete at settlement in that species. Karakiri et al. (1989) noted that the formation of accessory primordia "accompanies" the transition to a bottom-dwelling mode in plaice. If the formation of AP in California halibut "after metamorphosis" (Kramer, 1991) means "after eye migration is complete," then AP formation likely continues after settlement, because settling behavior of California halibut begins prior to initiation of eye migration (Gadomski et al., 1992).

Increased prominence of stress checks following enclosure may have been caused by a change in the otolith growth plane relative to the sectioning plane or by increased intensity of environmental cues that may induce check formation. Campana (1984b) correlated 15-day cycles of increment width and contrast in otoliths of intertidal starry flounder with similar cycles of tidally-induced temperature variation. Rosenberg (1982) described 14-day cycles of check formation in English sole (*Parophrys vetulus*), which first formed "at the beginning of the metamorphic period." An examination of Figure 2 of Rosenberg (1982) suggests that these checks were most obvious proximal to accessory primordia, as in Dover sole. The mean 15.3-day cycle of check formation observed in Dover sole may also be related to tidally induced temperature variation and larvae may be more strongly affected by these patterns following settlement, resulting in more prominent checks distal to the point of enclosure. However, the complex nature of rotational tidal currents in the vicinity of Dover sole nursery grounds precludes an evaluation of this hypothesis at present.

After accessory primordium formation, enclosure of growth from the central primordium, and formation of prominent checks, subsequent changes in larval otolith morphology were continuous, rather than discrete.

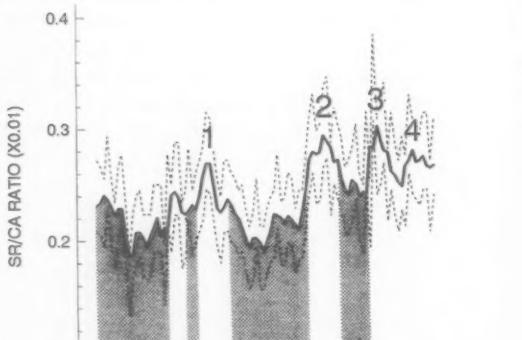

Figure 21

Relationship between Sr/Ca ratios and distance from boundary of enclosure towards edge in a 172.2-mm juvenile Dover sole, *Microstomus pacificus*, collected on 11 September 1989. The specimen had four post-settlement annuli and was prepared in transverse section. Solid line represents five-term running averages of Sr/Ca; dashed lines represent 2-σ measurement error, calculated as in Toole and Nielsen (1992); shaded areas represent opaque zones and unshaded areas represent translucent zones in the otoliths; and numbers above peaks represent post-settlement annuli.

There were no dramatic changes in otolith structure to mark the transition from Stage 3 to Stage 4.

Postmetamorphic juveniles Formation of the first post-settlement annulus occurred during the fall, several months after the transition from larval Stage 4 to juvenile Stage 5 that defines the end of metamorphosis (Markle et al., 1992). While the first and subsequent post-settlement annuli are useful for establishing the number of years since an individual settled and completed metamorphosis, they are not useful for determining either duration of the metamorphic period or the date associated with the end of metamorphosis. No other significant structural landmarks were associated with this stage.

Relation of otolith microchemistry to metamorphic stages

Premetamorphic larvae Within the clear central area formed during Stage 1, Dover sole otoliths exhibited high Sr/Ca levels relative to levels in adjacent opaque areas. Translucent zones of otoliths are rich in aragonite and have a poorly developed protein matrix, while opaque areas have higher protein concentrations (Dannevig, 1955; Williams and Bedford, 1974; Morales-Nin, 1987). Because organics are oxidized during beam exposure and 1:1 concentrations of CO_2 relative to cations are assumed in microprobe calculations (Toole and Nielsen, 1992), differences in concentration of organic materials could differentially affect accuracy of Ca and Sr measurements in translucent and opaque areas. However, Sr and Ca do not fractionate from one another in response to beam damage, so Sr/Ca ratios should not be affected by concentration of organics (Toole and Nielsen, 1992). Protein-poor areas of otoliths are generally associated with rapid growth (Dannevig, 1955; Williams and Bedford, 1974; Morales-Nin, 1987). However, many northeastern Pacific fishes, including adult Dover sole, form translucent zones (annuli) on otoliths during slow winter growth periods (Chilton and Beamish, 1982).

Sr/Ca ratios reflect the substitution of Sr for Ca in otolith aragonite, possibly owing to reduced ability to physiologically discriminate between Sr and Ca during biological precipitation (Radtke et al., 1990). The effect appears to be mediated through Sr concentration in the saccular endolymph, which in turn may be related to plasma protein levels (Kalish, 1989). Sr/Ca ratios have been described as inversely proportional to environmental temperature (Radtke, 1984, 1989; Townsend et al., 1989; Radtke et al., 1990) and growth rate (Sadovy and Severin, 1992), and directly proportional to age (Kalish, 1989), "stress" (Townsend et al., 1989), Sr concentration in ambient water (Kalish, 1990), and salinity of ambient water (Radtke et al., 1988). The latter two effects have only been documented under extreme conditions of seawater vs. freshwater residence. The effects of age described by Kalish (1989) were linked primarily to adults and may not apply to larvae and juveniles in this study.

Elevated Sr/Ca ratios in premetamorphic Dover sole otoliths could be related to a combination of low temperature, slow growth rate, and high "stress" (all of which may be expected to covary) if correlations observed in other studies apply to this species. However, confirmation of these effects in Stage-1 Dover sole is impractical, given available information. Temperatures experienced by premetamorphic larvae depend upon their distance offshore and depth, both of which are variable during this stage (Pearcy et al., 1977; Markle et al., 1992). Because periodicity of increment formation prior to AP formation has not been validated, growth rate cannot be determined with certainty. While it is possible that "stress" may be associated with developmental changes, the strong Sr/Ca spike approximately 48 μm from the central primordium appeared to occur subsequent to events such as hatching (at a length of ≈6 mm; Ahlstrom and Moser, 1975) and initiation of eye migration, caudal fin flexion, and growth

in body depth (at lengths of ≈10–15 mm; Pearcy et al., 1977; Markle et al., 1992), based on radii of otoliths from larvae of known lengths. (The radius of a 17-mm Dover sole larval otolith[2] was 38 µm and otolith radii of 20.6 and 30.1-mm larvae collected in the present study were 75 and 50 µm, respectively [Table 3].) Elevated Sr/Ca levels in the outer portion of the clear central area appeared to form near the termination of Stage 1, when larval morphology does not change (Markle et al., 1992).

Metamorphic larvae during Stage 2 and early Stage 3 Sr/Ca ratios declined in opaque otolith material presumably deposited during Stage 2 and early Stage 3. The decline either levelled prior to formation of the last AP or reached a minimum after enclosure of growth from the central primordium (Fig. 20A). Dover sole probably experienced progressively colder temperatures during this period as they moved from offshore waters onto the continental shelf and from the water column to the bottom (Pearcy et al., 1977; Landry et al., 1989; Markle et al., 1992). However, some larvae continue to move between the bottom and the water column, perhaps on a diel basis, throughout the metamorphic period (Markle et al., 1992), which would add further complexity to this pattern. Larvae appeared to lose mass and either did not grow in length or grew very little during this period (Markle et al., 1992). There was, however, a rapid increase in growth of otoliths, as evidenced by formation of accessory primordia with wide growth increments.

As with Sr/Ca ratios in otoliths of Stage-1 larvae, underlying causes of the observed pattern are unclear. If Sr/Ca ratios in Dover sole responded as in other species, decreasing temperature and slow somatic growth rate would be associated with increasing, rather than decreasing, Sr/Ca ratios. Otolith growth rate may be a better predictor of Sr/Ca ratio than somatic growth rate, and this may become apparent only when the two are uncoupled, as appears to occur during Stage 2 (Fig. 19).

Metamorphic larvae during Stages 3 and 4 Sr/Ca ratios remained low from formation of the last accessory primordium to formation of the first post-settlement annulus (Fig. 20B). Opaque otolith material formed during Stages 3 and 4, when most Dover sole were primarily, if not exclusively, on the bottom (Markle et al., 1992). Dover sole larvae increased in length and weight (Toole and Markle, unpubl. observations) and otolith length increased (Fig. 19) during this time, while bottom temperature decreased and nutrient levels increased following the onset of upwelling (Huyer et al., 1979; Landry et al., 1989). The correlation of low Sr/Ca ratios with rapid growth corresponds to observations of Sadovy and Severin (1992); the correlation of low Sr/Ca ratios with low temperature is contrary to observations of Radtke (1984, 1989), Townsend et al. (1989), and Radtke et al. (1990). Sadovy and Severin (1992) suggest that temperature was not the causative factor of Sr/Ca levels in previous studies; rather, it was an indicator of growth rate, which was positively correlated with temperature. For species such as Dover sole, which reside in upwelling systems where fastest growth occurs at cold temperatures, growth rate appears to over-ride temperature as a predictor of Sr/Ca ratios.

Postmetamorphic juveniles Microchemistry of otolith material deposited during Stage 5, prior to formation of the first post-settlement annulus, was identical to that formed following enclosure during Stages 3 and 4. The first and subsequent annuli were composed of translucent material deposited during the late fall and winter, with a high Sr/Ca ratio. During this period downwelling occurs, resulting in higher water temperature and lower nutrient concentrations than during the spring-summer upwelling season (Huyer et al., 1979; Landry et al., 1989). Kreuz et al. (1982) examined scales of adult Dover sole and noted that fastest growth occurred during the cold upwelling season, rather than during the warmer downwelling season, presumably because of higher productivity. The correlation of high Sr/Ca ratios and slow growth rate during formation of the first and subsequent post-settlement annuli is in agreement with observations of Sadovy and Severin (1992), as is the continued deposition of low Sr/Ca opaque material during subsequent seasons of rapid growth.

Summary of otolith landmarks

Several structural and chemical landmarks were correlated with metamorphic stages of Dover sole (Table 6). These landmarks were highly consistent among otoliths and, when coupled with information from daily growth increments, may be useful in determining timing of developmental and behavioral events associated with metamorphosis.

We conclude that the most consistent landmarks associated with the initiation event of metamorphosis (Markle et al., 1992), which includes movement of the left eye beyond the mid-dorsal ridge, are the transition from clear to opaque otolith material and formation of the first accessory primordium. Completion of eye migration apparently occurs between formation of the first and last AP, which encompasses a period of at

[2]J. Butler, S.W. Fisheries Science Center, NMFS, P.O. Box 271, La Jolla, CA 92038, pers. commun. 1992.

Table 6

Summary of larval and juvenile Dover sole, *Microstomus pacificus*, metamorphic stages (as described in text and Markle et al., 1992), structural landmarks on otoliths, chemical landmarks on otoliths, and habitat.

Stage	Structural landmarks	Chemical landmarks	Habitat
1	Translucent; all growth emanates from central primordium	Sr/Ca ratio high: 0.007–0.010 Spike and 0.004–0.006 "peak"	Pelagic; offshore from continental shelf
2	Opaque material; accessory primordia begin to form	Sr/Ca ratio declines from 0.004–0.006 to 0.002–0.003	Pelagic; moving inshore and towards bottom
End of 2 or beginning of 3	Opaque material; last accessory primordium forms; growth from accessory primordia encloses growth from central primordium	Sr/Ca ratio low, sometimes reaching minimum (≈0.002) near point of enclosure	First found on bottom; broad depth distribution on shelf and slope; also caught in midwater
3, 4, 5 Prior to first fall/winter on bottom	Opaque material; checks and left-right asymmetry become more prominent; rostrum forms	Sr/Ca ratio low (0.002–0.003)	Primarily benthic; narrow depth distribution on shelf; some Stage-3 and Stage-4 larvae also caught in midwater
5 Subsequent to first spring/summer on	Translucent material forming during fall/winter; opaque material forming during spring/summer	Sr/Ca ratio high (0.004–0.005) during fall/wiinter; low (0.002–0.003) during spring/summer	benthic

least 70 days in some individuals. The termination event of metamorphosis, as defined by Markle et al. (1992), is not associated with discrete structural or chemical signals.

We propose that the landmark most likely to correspond with the behavioral change of settlement from water column to benthos is enclosure of growth from the central primordium by growth from accessory primordia. In some individuals a minimal Sr/Ca value near 0.002 may also signal this event. This behavioral change is not instantaneous, because both Stage-3 and Stage-4 larvae were collected in midwater trawls. Markle et al. (1992) characterized settlement in Dover sole as a gradual process in which larvae make their way from an initial deep-water landing zone to a shallower nursery area. Enclosure of growth from the central primordium occurs near the beginning of Stage 3, when Dover sole larvae are first collected on the bottom in significant numbers. Thus, it is reasonable to expect that this is the point at which they first begin to spend a significant amount of time on the bottom.

Chronology of metamorphosis

Periodicity of increments emanating from the central primordium has not been validated, so assignment of dates to otolith landmarks formed during Stage 1 and Stage 2 can only be speculative. However, if increments are deposited approximately daily, then Stage-1 larvae reached a length of 20–30 mm in approximately 2–3 months, some Stage-1 larvae reached 46–50 mm in approximately 5–8 months, and most larvae reached the end of Stage 2 or beginning of Stage 3 about a year after increments first formed. These rates are approximately double those determined from seasonal collections of Dover sole larvae (Markle et al., 1992). Resolution of this discrepency must await validation of increment periodicity in premetamorphic larval otoliths.

Periodicity of growth increments was confirmed for Stage-3 and Stage-4 larvae and Stage-5 juveniles. In general, duration of these stages is in agreement with other studies. The duration of Stage 3 following enclosure averaged 28 days, although it extended at least 65 days for one individual. Because enclosure may oc-

cur during Stage 3, it is possible that total duration of Stage 3 is longer. Markle et al. (1992) suggested that, based on seasonal collections of Stage-3 and Stage-4 larvae, Stage 3 lasted approximately 45 days. Without a landmark for the Stage 3 to 4 transition, duration of Stage 4 could not be determined in this study. However, the combined period of Stages 3 and 4 could last up to 126 days, based on increments distal to enclosure of otoliths from Stage-4 larvae. Markle et al. (1992) observed one captive larva that took 43 days to progress through Stage 4, suggesting a combined duration through Stages 3 and 4 of about 90 days.

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Appendix

Origin of specimens examined for microstructure and microchemistry.

Stage	Number	Date(s)	Collection
1	1	January 1987	Midwater trawl; 300 km off Oregon; upper 200 m of water column
	2	March 1990	Midwater trawl; 75 and 532m bottom depth; upper 30 m of water column (Whipple, 1991[1])
	2	July 1991	Shrimp trawl; off Washington; 128 m depth
	1	October 1991	Shrimp trawl; off Oregon; ≈128 m depth
	Total: 6		
3	89	January 1989, March 1989, January 1990	Shrimp trawl with 6.4-mm liner; off Oregon; 55–377 m (Markle et al., 1992)
	14	March 1990	Midwater trawl; off northern California; bottom depths 73–1462 m; upper 110 m of water column (Whipple, 1991[1])
	Total: 103		
4	66	January 1989, March 1989, January 1990	Shrimp trawl with 6.4-mm liner; off Oregon; 40–170 m (Markle et al., 1992)
	16	March 1990	Midwater trawl; off northern California; bottom depths 33–91 m; upper 30 m of water column (Whipple, 1991[1])
	Total: 82		
5	220	January 1989, March 1989, January 1990	Shrimp trawl with 6.4-mm liner; off Oregon; 75–188 m (Markle et al., 1992)

Geographic variation and sexual dimorphism in the skull of the dusky dolphin, *Lagenorhynchus obscurus* (Gray, 1828)

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Abstract.—Variation in skulls of 415 dusky dolphins, *Lagenorhynchus obscurus*, was studied based on 37 standard cranial measurements and meristic variables and 28 nonmetrical characters (NMC) by using both bivariate and multivariate analyses. Geographic variation was analysed in mature skulls from central Peru (N=189), Chile (N=22), New Zealand (N=47) and southwestern Africa (N=40). Advanced fusion in the frontal-supraoccipital suture is the most reliable (95% efficiency) cranial criterion of sexual maturity; distal fusion in premaxillary and maxillary cannot be used. Sexual dimorphism, verified with t-tests and analysis of covariance, was encountered in only 6 of 37 measurements and in none of the NMC characters. Although skull size is the same, males have a wider rostrum and a longer temporal fossa than females. Analyses of covariance and t-tests indicated highly significant differences between geographic groups. A discriminant analysis successfully classified 96.5%, 91.7%, and 90.9% of skulls from respectively the Southeast Pacific (Peru and Chile pooled), New Zealand, and SW Africa. Such differentiation is considered of subspecific level. All methods indicated higher morphological similarity within the Peru/Chile and SW Africa/New Zealand grouped pairs than between the pairs. Most strikingly, skulls from New Zealand and SW Africa are on average 3.1 cm (8.5%) shorter than these from Peru and Chile, concordant with differences in body length. New Zealand specimens differ from SW African animals by smaller tooth size and a greater number of teeth. Nine cranial variables revealed significant variation between dusky dolphins from central Peru and northern Chile, but the degree of heterogeneity should be re-evaluated with a larger sample from Chile. NMC characters confirmed the trends mentioned but resolving power was limited; their use is not recommended as the principal method for subspecific discrimination. The Peruvian dusky dolphin is thought to represent the most ancestral (plesiomorph) form from which the others were derived through dispersion via the east-flowing Westwind Drift.

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The dusky dolphin, *Lagenorhynchus obscurus* (Gray, 1828), is mainly encountered in temperate coastal waters off Peru, Chile, Argentina, New Zealand, and South Africa (Gaskin, 1968; Webber, 1987; Van Waerebeek, 1992, a and b). Records, either specimens or sightings, are also available from Namibia[1] (Rose and Payne, 1991) and Angola[2].

This species was originally suggested to have a continuous circumpolar distribution (Brownell, 1965; Hershkovitz, 1966; Mörzer Bruyns, 1971); however, owing to its coast-dwelling habits many authors favor the notion of a disjunct distribution and existence of several isolated stocks (Brownell, 1974; Anonymous, 1975; Leatherwood and Reeves, 1983; Webber, 1987; Webber and Leatherwood, 1990). Nevertheless, evidence to support this hypothesis is lacking. Possible pelagic occurrence of dusky dolphins in the southern oceans and off many oceanic islands remains unknown. Specimen records are documented from the Campbell, Auckland, and Chatham Islands around New Zealand (Gaskin, 1968; Baker, 1983; Webber, 1987), the Falkland Islands (British Museum of Natural History specimens; Brownell, 1965) and from Gough Island (40°20'S, 10°00'W) on the mid-Atlantic Ridge[3]. Presence of dusky dolphins off southern Australia and Tasmania (reviewed by Webber, 1987; Van Waerebeek, 1993) and off the Kerguelen Islands (Robineau, 1991) could not be confirmed. Kasamatsu et al.[4] reported a single sighting from south of Madagascar but no photographs were taken and both the unusual (for *L. obscurus*) sea surface temperature (25°C) and offshore location solicits some caution. The sighting of a group of *Lagenorhynchus* sp. in deep tropical waters of the South Pacific, for instance, resulted in considerable debate before they were identified as *L. australis*, despite the availability of excellent photographs (Leatherwood et al., 1991).

Incidental fishing mortality occurs throughout the range of dusky dolphin and it is heavily exploited in Peru (Read et al., 1988; Van Waerebeek and Reyes, 1990, 1993) and, locally, off northern Chile (Van Waerebeek and

[1]P.B. Best, South African Museum, pers. commun. February 1991.

[2]P.B. Best, South African Museum, unpubl. photograph, identification confirmed by author.

[3]P.B. Best, South African Museum, unpubl. photographs, identification confirmed by the author.

[4]Kasamatsu, F., G. Joyce, P. Ensor, and J. Mermoz. 1990. Current occurrence of Cetacea in the southern hemisphere; results from the IWC/IDCR southern hemisphere minke whale assessment cruises, 1978/79–1987/88. Doc. SC/42/015 presented to the Int. Whal. Comm. Sci. Committee, 77 p.

Guerra, 1986[5]; Guerra et al., 1987). Population identification is needed so that breeding units can be managed separately. Cranial measurements have been used to discriminate populations in several delphinids including the common dolphin, *Delphinus delphis* (Evans, 1975, 1982; Heyning and Perrin[6]), bottlenose dolphin, *Tursiops truncatus* (Ross, 1977; Walker, 1981[7]), tucuxi, *Sotalia fluviatilis* (Borobia and Sergeant, 1989), pantropical spotted dolphin, *Stenella attenuata*, and spinner dolphin, *S. longirostris* (Perrin, 1975, a and b; Perrin et al., 1979; Schnell et al., 1982[8], 1985; Douglas et al., 1984).

In addition to skull measurements, non-metrical cranial characters have also been employed in population studies: harbor porpoises, *Phocoena phocoena* (Sjovold, 1977; Kinze, 1985, 1990[9]), spotted and spinner dolphins (Perrin et al., 1982[10]; Yablokov et al., 1983) and common dolphins (Perrin et al., 1988[11], 1989). The resolving power of non-metrical methods has been difficult to assess, however, because the identities of the dolphin stocks on which the technique was applied were debatable and results somewhat equivocal (Perrin et al., 1988[11], 1989). Also, non-metrical methods are more prone to observer bias and drift in character-state evaluation over time, especially if large samples are involved (e.g., Perrin et al., 1982[10], 1989).

Of the six extant species of *Lagenorhynchus*, geographic variation has been examined for the three northern hemisphere species: Pacific white-sided dolphin, *L. obliquidens* (Walker et al., 1986), Atlantic white-sided dolphin, *L. acutus*, and whitebeaked dolphin, *L. albirostris* (Mikkelsen, 1991). The purpose of this paper is to quantify sexual dimorphism and individual and geographical variation in skulls of *L. obscurus* from Peru, Chile, southwestern Africa (including South Africa, Namibia and Angola), and New Zealand utilizing material currently available in scientific collections. Evidence for separate dusky dolphin populations, as presented below, should urge agencies responsible for marine resource management in aforementioned coastal states to take special steps to ensure the conservation of *L. obscurus* on a stock by stock basis, rather than to evaluate the status of the species on a global scale.

Material and methods

Skulls were obtained by the author from animals recently killed in fishery operations as well as from museum specimens with widely variable degrees of documentation. Of more than 300 cranial specimens of *L. obscurus* from central Peru, collected along a coastal strip between Huacho (11°10'S) and San Andrés (13°50'S) in the period 1984 to 1990, 239 skulls were adequate for study. The majority of these were obtained from freshly butchered dusky dolphins at the wharves of Pucusana (12°30'S) and Cerro Azul (13°00'S); the rest were retrieved from decomposed remains at local fish-offal dumps or were beach-cast. When available, biological data, including sex, standard body length (SL) and reproductive status, were recorded for each specimen. Skulls were deposited at the Centro Peruano de Estudios Cetológicos, Pucusana, Peru; the Zoölogisch Museum of the University of Amsterdam; the Koninklijk Belgisch Instituut voor Natuurwetenschappen (Brussels); and at the U.S. National Museum of Natural History, Washington D.C. Surveys to collect stranded and by-caught specimens in northern Chile were carried out in collaboration with colleagues from the Instituto de Investigaciones Oceanológicas, Universidad de Antofagasta, Antofagasta, Chile (Van Waerebeek and Guerra, 1986[5]; Guerra et al., 1987).

The remainder of cranial material was located at the following institutions: the American Museum of Natural History, New York; British Museum (Natural History), London; Canterbury Museum and Canterbury University, Christchurch, New Zealand;

[5]Van Waerebeek, K. , and C. Guerra. 1986. Review of the distribution and status of the Burmeister's porpoise in Chile. Cetacean survey in II region of Chile, August–September 1986. Instituto de Investigaciones Oceanologicos, Universidad de Antofagasta, casilla 170, Antofagasta, Chile. Unpubl. Rep., 13 p.

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[8]Schnell, G. D., M. E. Douglas, and D. J. Hough. 1982. Geographic variation in morphology of spotted and spinner dophins (*Stenella attenuata* and *S. longirostris*) from the eastern tropical Pacific. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Southwest Fish. Sci. Cent., P.O. Box 271, La Jolla, CA 92038. Admin. Rep. LJ-82-15C, 213 p.

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[10]Perrin, W. F., A. V. Yablokov, and V. L. Cass. 1982. Preliminary report on the use of non-metrical skull characters to discriminate populations of pelagic dophins. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Southwest Fish. Sci. Cent., P.O. Box 271, La Jolla, CA 92038. Admin. Rep. LJ-82-14, 32 p.

[11]Perrin, W. F., A. V. Yablokov, J. Barlow, and M. V. Mina. 1988. The use of non-metrical skull characters for analyzing geographical variation in the common dolphin, *Delphinus delphis*. Part 1: Selection of characters. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Southwest Fish. Sci. Cent., P.O. Box 271, La Jolla, CA 92038. Admin. Rep. LJ-88-20, 24 p.

Departamento de Ciencias del Mar, Universidad Arturo Prat, Iquique, Chile; Museo Nacional de Historia Natural, Santiago de Chile; National Museum of New Zealand, Wellington; Otago Museum and Otago University, Dunedin, New Zealand; Port Elizabeth Museum, Port Elizabeth, and South African Museum, Cape Town, South Africa; U.S. National Museum of Natural History, Washington D.C.; and the Zoölogisch Museum, University of Amsterdam. Unfortunately, only a very few skeletal specimens from the SW Atlantic are available in Argentinian collections[12] and, for reasons of economy, it was impractical to examine these. Metrical and non-metrical data sets were collected by the author only, in order to reduce error and observer bias as a source of variation.

Cranial maturity

Juvenile specimens were excluded from the sample to reduce variability due to age. As many skull specimens did not have sexual maturity data, mature skulls were selected partly by using indirect criteria. To identify one or more cranial indicator(s) of sexual maturity in *L. obscurus*, selected sutures were inspected in 85 skulls (48 females, 37 males) from sexually mature specimens from Peru and in 31 skulls from subadults (large sexually immature specimens) from Peru, SW Africa, and New Zealand. Skulls which had been boiled (moderate heating for maceration excepted) or chemically treated were not included.

The degree of suture fusion between cranial elements was scored as: 0= no fusion, elements can be moved freely; 1= limited fusion, suture line clearly visible at all points; or 2= advanced fusion with partial obliteration of suture line. Occlusion of the pulp cavity in teeth (OCCL) was scored as: 0= wide open; 1= partially occluded; or 2= occlusion complete. The following seven cranial sutures were defined as follows:

SYM	symphysis mandibularis (between left and right ramus)
MXDIST	distal maxilla-premaxilla suture (rostral distal fusion)
PTPAL	pterygoid-palatine suture
PTBAS	pterygoid-basioccipital suture
LAC	lacrimal-maxilla/frontale suture
ZYG	zygomatic-parietal/exoccipital suture
FROC	frontal-supraoccipital suture.

The selection criteria for adult skulls were, in decreasing order of priority:

1 Skulls of sexual mature specimens as evidenced in males by presence of seminal fluid upon gross examination of the epididymis (Sergeant, 1962); in females by presence of at least one corpus luteum or corpus albicans in the ovaries or if lactating or pregnant, or both;

2 Advanced fusion (score 2) in the frontal-supraoccipital suture or advanced fusion in at least two of the other six defined cranial sutures.

The complete fusion of thoracic vertebral epiphyses, indicating physical maturity, was used to confirm cranial maturity of some specimens.

Metrical characters

Thirty-three standard metrical variables measured with vernier calipers to the nearest mm and four tooth counts, adapted from Perrin (1975a) and Schnell et al. (1982[8]) (see Table 3) were recorded for 381 dusky dolphin skulls. Standardized skewness and kurtosis were computed for all sample variables; if significant departure from normality was suspected, a Kolmogorov-Smirnov (K/S) one-sample test of the fit was applied. A ratio of variances test with 95% confidence limits was used to verify homogeneity of variance in all sample pairs. STATGRAPHICS version 4.2 programme (STSC Inc., 1989) was used on an AT286 personal computer for most statistical applications. The α=0.05 level was the criterion chosen for significance. All t-tests were two-sided. Owing to the large series of t-tests, a few significant results (false positives) should be expected to occur by chance alone.

Sexual dimorphism in cranial measurements and tooth counts was studied by means of t-tests and analysis of covariance (ANCOVA, with condylobasal length as covariate) in skulls of sexually mature females (N=59) and males (N=50) from central Peru. To permit pooling of sexes and utilization of cranially mature skulls of unknown sex, the few sexually dimorphic characters were not further considered in the analysis of geographic variation.

Seasonal variation was examined for in all variables by using a multifactor analysis of covariance (MANCOVA) with three-monthly seasons (January–March, April–June, etc.) and sex as classification variables.

Geographic variation was analysed for skulls from Peru (59 females, 50 males and 80 of unknown sex), New Zealand (N=47), SW Africa (N=40) and Chile (N=22). Both a one-way analysis of variance (ANOVA) and an ANCOVA with skull CBL as covariate and with geographic region as classification variable were utilized to assess among-group variation in skull size and shape. Next, most morphologically similar group pairs,

[12]R. N. P. Goodall, Centro Austral de Investigaciones Cientificas, Ushuaia. Pers. commun. July 1988.

namely Peru/Chile and SW Africa/New Zealand were compared by means of *t*-tests to identify discriminating variables.

Discriminant analyses (DISCRIM) were performed on samples limited to specimens with a complete set of measurements. This sample included 135 skulls from Peru, 24 from New Zealand, 22 from SW Africa and 7 from Chile. A first DISCRIM with 21 characters maximized discrimination between geographic groups. Subsequently, a series of discriminant analyses was executed by considering various combinations of a small number of highly variable cranial characters so that an optimum segregation between groups was obtained. These discriminant functions can be used tentatively to assign undocumented museum skulls to respective populations. It must be cautioned however that no specimens from Argentina have been examined and the discriminant locus of that population remains unknown.

Non-metrical characters

Initially, 35 non-metrical cranial characters (NMC), following Perrin et al. (1982[10]), were determined, but serious problems with score repeatability over time reduced the number of usable NMC to 27 (Table 1 and Fig. 1). Similar problems have been reported for the common dolphin by Perrin et al. (1988[11], 1989).

Analysis of sexual dimorphism was limited to skulls of sexually mature specimens from central Peru (N=109), eliminating influences of growth and geography. Sexually monomorphic characters (the full set, see Results) were then tested for dependence on cranial maturity status in 173 mature and 48 immature skulls. Geographic variation in non-metrical traits among the four geographic areas was studied after eliminating variables influenced by sex and maturity. All significance testing of variation in NMC was done by means of chi-square contingency analyses. Tooth counts were compared by *t*-tests.

Table 1

Non-metrical cranial characters utilized in present study (from Perrin et al., 1982[9]); see Figure 1.

Character 1L/1R (left/right).	Position of the foramen (A in Figure 1) in the premaxillary at the base of the prenarial triangle, relative to the level of the anteriormost projection of the antorbital processes. Codes: 1=at; 2=ahead; 3=behind the level.
Character 2L/2R.	Position of the anteriormost large foramina (B) in the maxillary relative to the level of the anteriormost projection of the antorbital processes. Codes: 1=at; 2=ahead; 3=behind the level.
Character 3.	Asymmetry of position of the two anteriormost large maxillary foramina (B). Codes: 1=symmetrical; 2=left foramen more anteriorly placed; 3=right foramen more anterior.
Character 5L/5R.	Number of small foramina (C) in the maxillary anterior to the anteriormost of the large foramina. Codes: 1=one foramen; 2=two foramina;3 etc.; 6=no foramina.
Character 6L/6R.	Number of foramina (D) in the maxillary behind a line at the level of the anterior edge of the external nares and perpendicular to the long axis of the skull. Codes: 1=1 foramen; 2=2 foramina; 3 etc.; 6= no foramina.
Character 9L/9R.	Contact between maxillary and occipital, at point where occipital crest intersects margin of temporal fossa (E). Codes: 1=contact (or space of <1mm); 2=no contact.
Character 10.	Contact between premaxillary and nasal, on right side (F). Codes: 1=contact (or space of <1mm); 2=no contact.
Character 12L/12R.	Presence of an antorbital notch (G). Codes: 1=notch present; 2=not present.
Character 13.	Composition of the antorbital process, in lateral view, on left side (H). Codes: 1=formed by lacrimal; 2=formed by lacrimal and frontal; 3=formed by lacrimal and maxillary.
Character 16.	Accessory foramen above foramen magnum (I). Codes: 1=present; 2=absent.
Character 17.	Clear notch in upper margin of foramen magnum (J). Codes: 1=present; 2=absent.
Character 18L/18R.	Fenestrations in occipitals, near foramen magnum and in exoccipital region (K). Codes: 1=one fenestration; 2=two; 3 etc.; 6=no fenestrations.
Character 19.	Fenestrations in region of occipital swellings (L). Codes: 1=one fenestration; 2=two etc.; 6= no fenestration.
Character 23.	Posterior contact between pterygoid hamuli (M). Codes: 1=open (gap >1mm); 2=closed.
Character 24.	Anterior contact between hamuli (N). Codes: 1=open (gap>1mm); 2=closed.
Character 25.	Spine of palatines visible in anterior gap between hamuli (O). Codes: 1=visible; 2= not visible.
Character 30.	Mesial fenestration in basisphenoid (R). Codes: 1=foramen present; 2=absent.
Character 33L/33R.	Number of mental foramina. Codes: 1=1; 2=2; etc.
Character 34L/34R.	Arrangement of mental foramina. Codes: 1=foramina lie along a simple curve; 2=foramina do not lie along a simple curve.

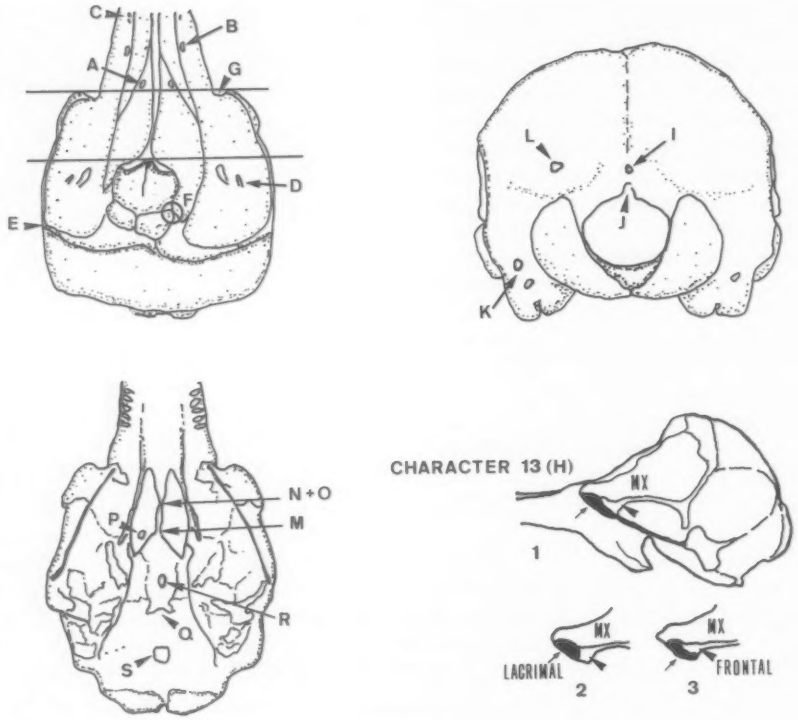

Figure 1
Non–metrical cranial characters utilized in this study following Perrin et al. (1982). Letters refer to Table 1.

Results

Cranial maturity

Relationships between the degree of fusion in cranial sutures and sexual maturity for *L. obscurus* are shown in Table 2. Less than 25% of the adults exhibited advanced rostral distal fusion (MXDIST=2) which, as in *L. obliquidens* (see Walker et al., 1986), eliminates MXDIST as a selection criterion for adult skulls in this species (too many useful skulls would be rejected). However, three other sutures, frontale-supraoccipitale (FROC), zygomatico-parietale/exoccipitale (ZYG), and lacrimale-maxilla/frontale (LAC), had advanced fusion (state=2) in 95%, 89%, and 85%, respectively of the sexually mature specimens. Equally important, only two sexually immature specimens from New Zealand showed full fusion in any of these sutures: one was a pubescent female with large follicles (ZM39957) and the other, an unusually large female without ovarian corpora (ZM39561, 187 cm). Moreover, based on overall size, those skulls fitted into the adult series. Skulls with at least some fusion (state 1 or 2) in either MXDIST, SYM, or PTPAL are very likely from sexually mature dolphins (see Table 2).

Metrical characters

Individual variation Summary statistics for cranial characters in adult female and male dusky dolphins of central Peru are presented in Table 3. Of 74 characters tested, only one (upper left tooth count, for females) departed significantly from normality (Kolmogorov-Smirnov, P=0.017), which could be attributed to chance fluctuations. Homogeneity of variance (95% confidence, TWOSAM, STSC Inc., 1989) was confirmed for all sex-paired characters.

Statistics summarizing individual variation in cranial characters are given also for mature dusky dolphins of northern Chile, southwestern Africa and New Zealand (Table 3). No significant departures from nor-

Table 2

Percent frequencies, rounded to nearest integer, for degrees of fusion seen in cranial sutures of *Lagenorhynchus obscurus* specimens of known sexual maturity status (sexes pooled). Mature animals are all from Peruvian waters; subadults include specimens from Peru, southwestern Africa, and New Zealand. States of fusion are: 0= unfused; 1= some fusion; 2= advanced fusion. Cranial sutures are defined in text.

Suture	Subadults (%) Fusion state				Mature (%) Fusion state			
	N	0	1	2	*N*	0	1	2
SYM	31	94	3	3	71	47	28	25
MXDIST	29	100	0	0	77	23	53	23
PTPAL	30	97	3	0	85	4	28	68
PTBAS	30	80	20	0	85	4	28	68
LAC	30	50	50	0	85	0	15	85
ZYG	31	81	13	6	85	1	9	89
FROC	31	45	48	6	85	0	5	95
OCCL	—	—	—	—	90	4	41	54

mality were encountered in any of the characters (K/S, $P>0.5$).

Sexual dimorphism The overall size of the adult dusky dolphin skull, based on condylobasal length and zygomatic width, is equal in males and females (t-tests, $P>0.50$; Tables 3 and 4). Highly significant sexual dimorphism (t-tests, $P<0.005$) was found in the width of the rostrum over most of its length (RW60, RW1/4, RW1/2, PMX1/2, RW3/4) and in the length of the temporal fossa; males had consistently higher mean widths. Analyses of covariance showed sexual dimorphism in exactly the same variables and with very similar significance levels as in the two-sided t-tests, indicating proportional differences (shape). However, it is uncertain whether the observed, small dissimilarities have biological meaning, particularly as there is wide overlap in ranges. I was unable to produce a satisfactory model to predict the sex of an unknown animal through stepwise variable selection in a multiple regression (see Douglas et al., 1986) exactly because of those subtle deviations.

Twenty-four of 36 characters showed significant correlations with the condylobased length (CBL) covariate, including five of the six dimorphic characters (Table 4), implying that much of the variability is explained by variation in CBL. Eliminating dimorphic characters from the geographic analysis therefore entails little loss of information.

Seasonal variation An analysis of seasonal variation was feasible only for Peruvian specimens. Skull size (CBL) was not seasonally dependent (MANCOVA, $F=1.45$, df=3, $P=0.23$) but six other skull characters (RW60, RW1/4, APL, RAL, TCUL, and TCUR) demonstrated seasonal variation at a low level of significance (MANCOVA, $0.05 < P < 0.02$). However, at least in the case of RW60, RW1/4, and RAL, significant interactions due to sexual dimorphism in these measurements seemed to be the cause (P-values for 2-factor interactions were respectively 0.039, 0.047, and 0.037). In APL, TCUL, and TCUR, which are sexually monomorphic characters (see above), there were no sex/season interactions ($P>0.50$). The significance in these few cases could be due to chance in view of the large number of t-tests. This is supported by the results of a series of Runs tests (Runs above and below median, Runs up and down, STSC Inc., 1989) on chronologically ordered CBL, tooth counts and tooth widths. These results did not suggest any non-randomness ($Z>0.05$ for all tests).

Geographic variation Exclusion of the few sexually dimorphic characters allowed combining samples of males and females and the use of cranially mature skulls of unknown sex. Highly significant differences between geographic regions were confirmed for 29 of 31 cranial characters (for all ANOVA, $P<0.005$ and mostly $P<0.0001$; Table 5). Tympanic bulla width (BW) proved divergent between groups but with less confidence (ANOVA, $F=4.1$, df=3,117, $P=0.019$) and no difference could be detected for periotic length (ANOVA, $F=0.24$, df=3,201, $P=0.87$). Variance generally was homogeneous (Cohran's C-test and Bartlett's test, $P>0.01$ and mostly $P>0.05$); exceptions include TCLL (Cohran's, $P=0.006$) and PEL (Cohran's, $P=0.003$; Bartlett's, $P=0.009$).

ANCOVA analyses revealed discernible variation in 16 of 30 skull characters which can not be attributed to divergences in skull size alone but are due to proportional differences (i.e., shape); 11 characters were highly significantly different between geographic groups ($P<0.01$, Table 5). Means and 95% confidence intervals are plotted for each variable against geographic region (Fig. 2). The most striking element is the overwhelming difference in skull size, reflected in most measurements, grouping skulls from Peru and Chile and specimens from New Zealand and Southwest Africa. Skulls of dusky dolphins from the latter pair-group have, on average, a CBL about 3 cm shorter than skulls from Peru and Chile.

Table 3

Skull measurements (in mm) and tooth counts of mature dusky dolphins *Lagenorhynchus obscurus* from different populations. Sexes are pooled except for Peru; tooth counts of Chilean specimens include some juvenile specimens. Skull selection criteria are explained in text.

Zealand	Peru: Females				Peru: Males				Chile				SW Africa				New			
Variable	N	Mean	SD	Range	N	Mean	SD	Range	N	Mean	SD	Range	N	Mean	SD	Range	N	Mean	SD	Range
Condylobasal length (CBL)	59	398.4	12.6	368–426	49	396.9	11.3	372–420	14	394.9	9.57	377–410	34	365.8	11.0	342–386	41	367.0	10.9	344–388
Rostrum length (RL)	59	221.8	9.19	198–224	49	219.5	8.29	202–241	13	220.8	9.41	208–235	36	198.8	7.0	178–214	42	198.4	9.0	178–215
Rostrum width at base (RWB)	59	101.1	4.63	87–112	50	101.5	4.84	92–113	21	100.5	4.71	89–109	39	97.7	5.4	75–107	45	97.2	4.6	89–108
R. width at 60 mm (RW60)	59	74.7	3.35	68–82	50	76.9	3.62	68–84	21	74.8	3.47	69–80	40	67.8	3.2	59–75	45	66.7	3.4	61–77
R. width at 1/4 length (RW1/4)	58	75.5	3.21	69–83	49	77.8	3.55	69–85	16	76.1	3.36	70–82	36	70.1	2.8	64–76	41	68.6	3.3	63–78
R. width at 1/2 length (RW1/2)	59	65.3	2.95	59–72	49	67.5	3.4	59–75	16	66.3	3.93	58–71	36	60.2	2.9	54–68	42	59.2	3.5	53–67
Premax. width at 1/2 length (PMX1/2)	59	35.5	2.44	29–41	49	37.2	2.68	32–43	16	36.7	2.55	33–41	36	34.1	2.1	30–40	42	32.8	2.5	27–39
R. width at 3/4 length (RW3/4)	59	48.2	2.44	42–54	49	50.3	2.78	45–57	16	49.4	4.45	41–55	33	45.5	3.0	39–53	39	44.9	3.1	39–50
Rostrum tip to ext. nares (RTEN)	58	264.1	11.0	232–228	46	262.2	9.33	245–284	14	259.9	12.8	229–277	35	237.3	8.0	214–250	40	239.8	12.1	198–261
Rostrum tip to int. nares (RTIN)	58	261.5	11.2	232–228	49	260.0	10.8	240–283	10	259.6	8.98	246–274	28	235.1	8.0	219–250	35	238.1	9.2	214–254
Preorbital width (PROW)	57	166.9	4.89	151–117	50	167.6	4.19	159–178	19	164.3	6.0	154–178	32	159	4.7	151–169	43	158.9	5.7	145–171
Postorbital width (POOW)	57	184.4	5.54	168–119	50	184.8	4.97	176–198	16	184.0	5.98	175–195	33	175.4	4.8	164–186	40	175.8	5.4	166–190
Zygomatic width (ZYW)	57	187.6	5.17	178–119	50	188.3	5.59	179–202	17	187.2	5.85	178–199	34	177.8	5.2	165–191	44	177.7	5.4	167–194
Parietal width (PARW)	59	152.6	6.11	137–116	50	153.9	5.96	135–166	15	154.6	6.16	146–166	37	150.8	5.1	136–160	46	150.2	5.5	138–160
Greatest width premax. (GWPMX)	59	78.0	2.98	72–85	50	77.3	2.76	72–83	22	78.2	3.16	74–86	40	76.0	3.2	68–85	47	75.9	3.6	67–85
External nares width (ENW)	59	55.4	2.56	48–62	50	54.5	2.31	50–59	22	54.2	2.22	50–59	39	54.4	2.6	47–61	47	53.2	2.6	49–61
Temporal fossa length (TFL)	59	71.4	3.87	62–80	50	74.2	4.9	63–86	13	74.0	3.51	66–79	36	69.6	4.4	59–78	47	69.6	4.0	60–76
Temporal fossa width (TFW)	59	52.4	3.41	43–62	50	52.8	3.98	44–65	16	52.5	3.08	48–57	37	51.1	3.8	41–60	47	49.5	3.0	43–56
Orbit length (ORL)	58	52.2	2.35	48–58	50	52.1	2.32	48–59	18	52.8	2.24	47–56	35	51.0	2.4	45–56	46	49.8	2.1	45–56
Antorbital process length (APL)	58	38.1	2.89	32–45	50	38.7	2.76	33–46	17	36.3	2.11	32–40	37	34.8	2.8	29–41	46	35.5	3.3	26–42
Internal nares width (INW)	59	57.7	3.04	51–65	50	57.7	2.81	52–64	18	56.9	3.07	53–63	37	52.4	2.5	47–57	44	53.7	2.7	48–60
Pterygold length (PTEL)	55	65.5	4.3	57–77	50	64.4	4.0	56–74	11	62.1	2.74	57–66	30	59.0	4.0	51–73	35	57.7	4.0	49–66
Upper tooth row length (UTRL)	59	192.9	8.38	171–221	49	190.1	7.58	171–207	13	191.2	7.25	180–204	32	171.2	6.4	153–182	43	170.2	8.2	151–183
Lower tooth row length (LTRL)	55	192.7	7.54	171–220	47	190.9	8.16	171–210	11	183.5	9.09	172–195	34	172.4	7.5	159–187	41	170.7	8.5	143–191
Ramus length (RAL)	55	335.4	9.97	312–360	47	334.5	11.3	309–360	11	327.9	10.52	311–346	32	308.6	10.1	286–330	40	306.3	9.6	284–326
Ramus height (RAH)	54	69.2	2.95	63–80	47	69.3	2.65	62–74	9	68.8	1.92	66–71	31	65.1	2.6	60–71	40	65.3	2.6	60–71
Tooth count upper left (TCUL)	58	32.0	1.59	29–37	49	31.8	1.73	29–36	17	30.9	2.07	26–34	29	30.4	1.6	28–34	36	31.9	1.8	28–37
Tooth count upper right (TCUR)	58	32.0	1.74	29–39	49	31.8	1.69	29–36	14	30.6	1.9	26–33	30	30.2	1.7	27–33	37	32.0	1.6	29–36
Tooth count lower left (TCLL)	55	31.1	2.06	26–37	47	31.3	1.58	28–36	14	30.2	1.97	28–35	31	29.6	1.5	26–32	40	31.5	2.2	28–39
Tooth count lower right (TCLR)	55	30.8	2.14	26–37	47	31.0	1.41	28–36	17	30.4	2.26	27–35	32	29.6	1.5	27–34	40	31.4	2.2	27–37
Tooth width at mid–jaw (TW)	55	4.4	0.36	3.7–5.1	49	4.4	0.35	3.6–5.5	16	4.3	0.28	3.7–4.7	36	4.18	0.37	3.3–5.2	32	3.65	0.35	2.9–4.3
Maximum bulla length (BL)	55	35.4	1.19	33–39	49	35.4	1.38	33–38	5	34.6	0.89	34–36	30	34.0	1.1	32–36	18	33.9	1.2	31.5–36
Maximum bulla width (BW)	20	19.8	0.69	19–21.5	20	19.7	0.85	18.5–21.5	—	—	—	—	30	19.5	0.6	18.5–20.5	15	19.3	0.7	18–20.5
Height braincase (HBR)	58	109.5	4.45	101–119	50	110.3	4.45	100–119	19	109.9	5.25	103–21	37	107.1	4.0	99–119	45	105.0	4.8	93–115
Length braincase (LBR)	58	126.9	4.6	113–138	50	127.9	4.04	118–134	19	125.9	3.93	117–133	37	122.5	4.3	113–131	45	121.6	4.2	112–131
Periotic length (PEL)	54	30.2	1.16	28–33	49	30.33	1.06	27–32	6	30.5	1.64	28–33	27	30.0	1.2	28–32	12	30.4	1.3	28–32
Height crest (HCR)	52	7.66	2.13	3.9–14.5	48	7.17	1.77	4–12.5	—	—	—	—	30	5.5	2.1	1–10	41	7.2	2.1	3.5–13

Table 4

Results of two-sided *t*-tests and multifactor analysis of covariance (MANCOVA) to determine sexual dimorphism and seasonal variation (see text) in skull measurements of mature female (N=59) and male (N=50) *Lagenorhynchus obscurus* from Peru. Condylobasal length (CBL) is used as the covariate. Measurements are defined in the text.

	Two-sided *t*-test		MANCOVA			
			Covariate (CBL)		Factor 1 (sex)	
Variable	*t*	*P*	*F*-ratio	*P*	*F*-ratio	*P*
CBL	0.63	0.53	—	—	0.37	0.55
RL	1.33	0.19	328	<0.0001	2.0	0.16
RWB	0.49	0.62	0.43	0.52	0.32	0.58
RW60	3.32	<0.002	12.3	<0.001	12.1	<0.001
RW1/4	3.62	<0.0005	4.87	<0.05	12.8	0.0005
RW1/2	3.58	<0.0001	12.3	<0.0001	13.1	0.0005
PMX1/2	3.59	<0.0001	0.29	0.59	10.4	0.002
RW3/4	4.2	<0.0001	8.72	<0.005	18.7	<0.0001
RTEN	0.93	0.36	201.5	<0.0001	0.001	0.98
RTIN	0.74	0.46	470.4	<0.0001	0.29	0.59
PROW	0.78	0.44	13.3	<0.0005	0.71	0.41
POOW	0.31	0.75	12.38	<0.001	0.04	0.84
ZYW	0.64	0.52	16.8	0.0001	0.62	0.44
PARW	1.17	0.24	2.41	.012	1.40	0.24
GWPMX	1.29	0.20	1.68	0.20	1.54	0.22
ENW	1.74	0.09	0.91	0.35	2.98	0.09
TFL	3.36	<0.005	7.35	<0.01	10.54	0.002
TFW	0.61	0.54	0.05	0.94	0.13	0.72
ORL	0.19	0.85	35.1	<0.0001	0.63	0.44
APL	1.26	0.21	2.70	0.10	1.54	0.22
INW	0.10	0.92	3.31	0.07	0.01	0.92
PTEL	1.39	0.17	30.5	<0.0001	1.1	0.30
UTRL	1.75	0.08	281.5	<0.0001	3.56	0.06
LTRL	1.10	0.27	135.1	<0.0001	0.26	0.61
RAL	0.42	0.67	386.2	<0.0001	0.54	0.47
RAH	0.06	0.95	13.4	<0.0005	0.07	0.79
TCUL	0.76	0.45	4.79	<0.05	1.41	0.24
TCUR	0.65	0.51	4.73	<0.05	0.95	0.34
TCLL	0.53	0.60	1.42	0.24	0.08	0.78
TCLR	0.54	0.60	1.32	0.25	0.09	0.77
TW	0.08	0.94	4.63	<0.05	0.36	0.55
BL	0.05	0.96	15.6	0.0002	0.003	0.96
BW	0.41	0.69	0.26	0.62	0.008	0.93
HBR	0.95	0.35	5.14	<0.05	1.72	0.19
LBR	1.20	0.23	42.3	<0.0001	2.55	0.11
PEL	0.64	0.52	29.1	<0.0001	0.84	0.37
HCR	1.26	0.21	2.67	0.11	1.98	0.16

Southwest African versus New Zealand population Results of two-sided *t*-tests comparing adult skulls from southwest Africa and New Zealand are presented in Table 6. Skulls from these regions do not differ in size, as indicated by condylobasal length (t=0.48, P=0.63) and zygomatic width (t=–0.074, P=0.94). Nevertheless, the two populations are strikingly divergent in dentition. Mean tooth width for New Zealand dusky dolphins (3.56 mm, SD=0.35, N=32) is highly significantly smaller (t=–7.14, P<0.0001) than in SW African dusky dolphins (4.18 mm, SD=0.37, N=36); the difference is visually obvious even from a quick inspection of skulls. In addition, New Zealand crania have on average 1.5–1.8 teeth more in each row than African skulls (two-sided *t*-tests, P<0.001). Maximum tooth counts in the four rows range 32–34 (SA) and 36–39 (NZ). Finally, SW African crania have a significantly lower supraoccipital crest (t=3.43, P=0.005), slightly wider external nares and temporal fossa and, possibly, a somewhat higher braincase (0.05<P<0.01) than the New Zealand specimens.

Peruvian versus Chilean population Dusky dolphin crania from central Peru and northern Chile showed no meaningful overall size differences, as indicated by CBL and ZYG (Table 6). The antorbital process (APL) and the lower tooth row (LTRL) however were very significantly shorter (*t*-test, P<0.005) and mean tooth counts were lower in Chilean animals. Small but statistically discernible deviations (P<0.05) were detected also in PROW, PTEL, and RAL. These data suggest that at least some genetic distance exists between *L. obscurus* stocks from central Peru and northern Chile, but it is recommended that the sample size for Chile be increased before definite conclusions be made.

Multivariate analysis Results of the discriminant analyses (DISCRIM) with a maximum of characters (21) fully confirm findings from bivariate techniques (Table 7); scores on first and second axis are shown in Figure 3. The first discriminant function differentiated well between population pairs Peru/Chile and New Zealand/SW Africa; the second function largely separated SW Africa and New Zealand stocks (Fig.4), whereas the third function projected the Chile group at the positive extreme of the z-axis (see group centroids, Table 7). The discriminant functions successfully predicted the actual group most of the time, i.e., from 85.7% of cases in Chile to 91.7% in New Zealand. When Chilean and Peruvian animals were combined into a single "Southeast Pacific stock," 96.5% (137 of 142) of these were correctly allocated. Only two of 24 (8.3%) New Zealand skulls were misclassified as SW African but they were never mistaken for SE Pacific animals. Two skulls out of 22 (9.2%) from SW Africa were erroneously assigned, one each to the New Zealand and Peru stocks. The highest percentages of misclassifications (10.4% and 14.3%) occurred among

Table 5

Geographic variation in cranial characters of mature *Lagenorhynchus obscurus* (N = 298) from Peru, Chile, southwestern Africa and New Zealand. Sexes were pooled after removal of sexually dimorphic variables (see Table 3). NS = not significant, $P>0.05$.

	ANOVA			ANCOVA	
Variable	*F*–ratio	df	*P*	*F*–ratio	*P*
CBL	120.2	3,275	<0.0001	—	—
RL	118.3	3,274	<0.0001	2.48	NS
RWB	10.1	3,287	<0.0001	0.66	NS
RTEN	63.5	3,261	<0.0001	0.83	NS
RTIN	82.4	3,245	<0.0001	1.05	NS
PROW	40.0	3,273	<0.0001	2.46	NS
POOW	44.6	3,264	<0.0001	3.78	<0.02
ZYW	54.5	3,276	<0.0001	4.14	<0.01
PARW	6.88	3,282	<0.0005	0.87	NS
GWPMX	4.39	3,294	<0.005	1.99	NS
ENW	5.35	3,293	<0.0005	1.66	NS
TFW	13.2	3,285	<0.0001	3.65	<0.02
ORL	18.0	3,280	<0.0001	3.31	<0.05
APL	22.9	3,280	<0.0001	3.38	<0.05
INW	34.1	3,282	<0.0001	4.82	<0.005
PTEL	33.5	3,242	<0.0001	2.89	<0.05
UTRL	122.0	3,270	<0.0001	4.33	<0.01
LTRL	109.3	3,242	<0.001	8.92	<0.001
RAL	118.3	3,239	<0.0001	6.18	<0.001
RAH	34.9	3,232	<0.0001	0.14	NS
TCUL	7.4	3,256	<0.0005	6.68	<0.0005
TCUR	9.65	3,256	<0.0001	7.74	<0.0005
TCLL	11.7	3,236	<0.0001	8.85	<0.0001
TCLR	10.1	3,237	<0.0001	8.25	<0.0001
TW	50.3	3,260	<0.0001	25.2	<0.0001
BL	13.9	3,208	<0.0001	0.35	NS
BW	4.1	3,117	<0.02	0.70	NS
HBR	19.5	3,285	<0.0001	4.59	<0.005
LBR	28.4	3,285	<0.0001	1.08	NS
PEL	0.24	3,201	NS	2.31	NS
HCR	6.33	3,235	<0.0005	3.47	NS

Peruvian and Chilean crania, explainable by the relatively greater morphological similarity and geographic proximity. Overall there were successfully identified to geographical population 164 out of 188 (87.2%) of the skulls, increasing to 179 out of 188 (95.2%) when skulls from Chile and Peru were pooled.

Six characters with high discriminatory power were identified from the standardized discriminant function coefficients, *t*-tests and ANOVA's and an additional discriminant analysis was performed using those variables (Table 8). Classification results and the discriminant function plot (Fig. 4) demonstrate that specimen prediction remains fairly effective (143 of 188 or 76.1% and 171 out of 188 or 91.0% when the SE Pacific sample is pooled as one group).

Non-metrical characters

Tooth counts—As expected, tooth counts derived from skulls were consistently greater ($P<0.0001$) than counts made on heads in the flesh because small, apical teeth frequently do not erupt from the gums (see Van Waerebeek, 1992b). Differences of mean tooth counts between skull and fresh specimens varied from 2.29 to 2.67 per jaw arcade. Considering confidence intervals, the best estimate (closest integer) for each of the four arcades was two more teeth visible in skulls than in heads in the flesh.

Tooth counts are bilaterally symmetrical in upper and lower jaws and for all sample pairs (*t*-tests, $P>0.16$). The upper jaw has significantly more teeth than the lower jaw, (one-sided *t*-tests; $P<0.005$ in all cases); confidence intervals included one (tooth) as the largest integer but not zero.

Sexual dimorphism—The 28 non-metrical cranial characters (NMC) tested are, without exception, independent of sex (chi-square contingency tests, $P>0.05$). Sexual dimorphism neither exists in the number of teeth in Peruvian dusky dolphins, both as counted from skulls and from fresh animals (*t*-tests, $P>0.25$). It is considered safe to assume that this is generally true for the other populations of *L. obscurus* and therefore the sexual factor is disregarded in the subsequent analyses.

Developmental variation—Twenty-two of the 28 NMC characters tested (78.6%) in Peruvian dusky dolphins are independent of maturity status (Table 9). Variables which may be correlated with age to some degree (chi-square, $P<0.05$) include position of the left premaxillary foramen at the base of the prenarial triangle (1L), presence of fenestration(s) in occipitals (18L) or basisphenoid (30), visibility of palatine spine (25), and the number of mental foramina (33L). At least one of these is expected to be due to chance fluctuation ($\alpha=0.05$). The more frequent occurrence of a notch in the upper margin of the foramen magnum (17) in immature animals as compared to adults proved highly significant (chi-square=8.83, $P<0.005$).

Geographic variation—The six growth dependent characters were removed from the data set before variation between populations was tested. By doing this, samples available for geographic variation analysis were greatly enhanced as juvenile specimens (but not neonates) could be included. Table 10 lists absolute

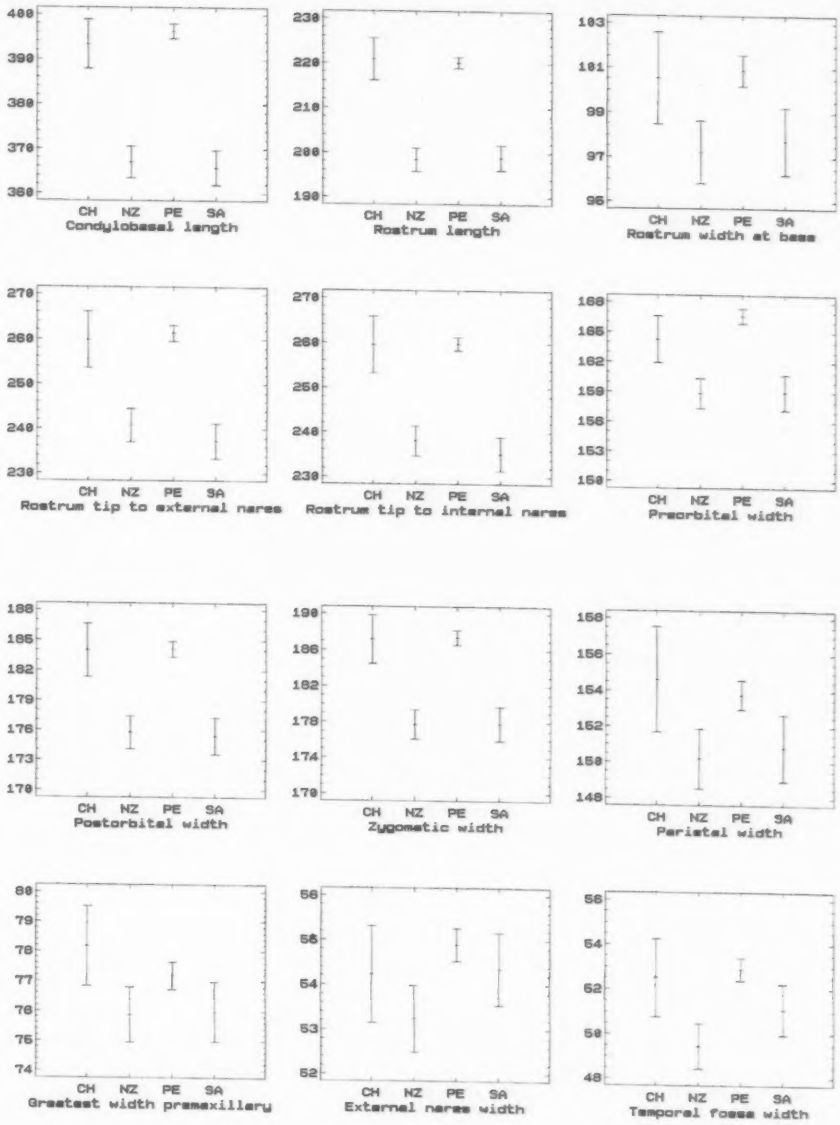

Figure 2

Comparison of means in cranial measurements (in mm) and meristics for adult *Lagenorhynchus obscurus* from Chile (CH), New Zealand (NZ), Peru (PE) and southwestern Africa (SA). Error bars are 95% confidence intervals for means.

frequencies of NMC character states in skulls from Peru (N=221), Chile (N=40), New Zealand (N=69), and southwestern Africa (N=65). Surprisingly, only three characters out of 22 (14%) were found to demonstrate significant differences between geographic groups (chi-square contingency analysis; see Table 10). These are relative position of the foramen in the right premaxillary at the base of the prenarial triangle (variable 1R,

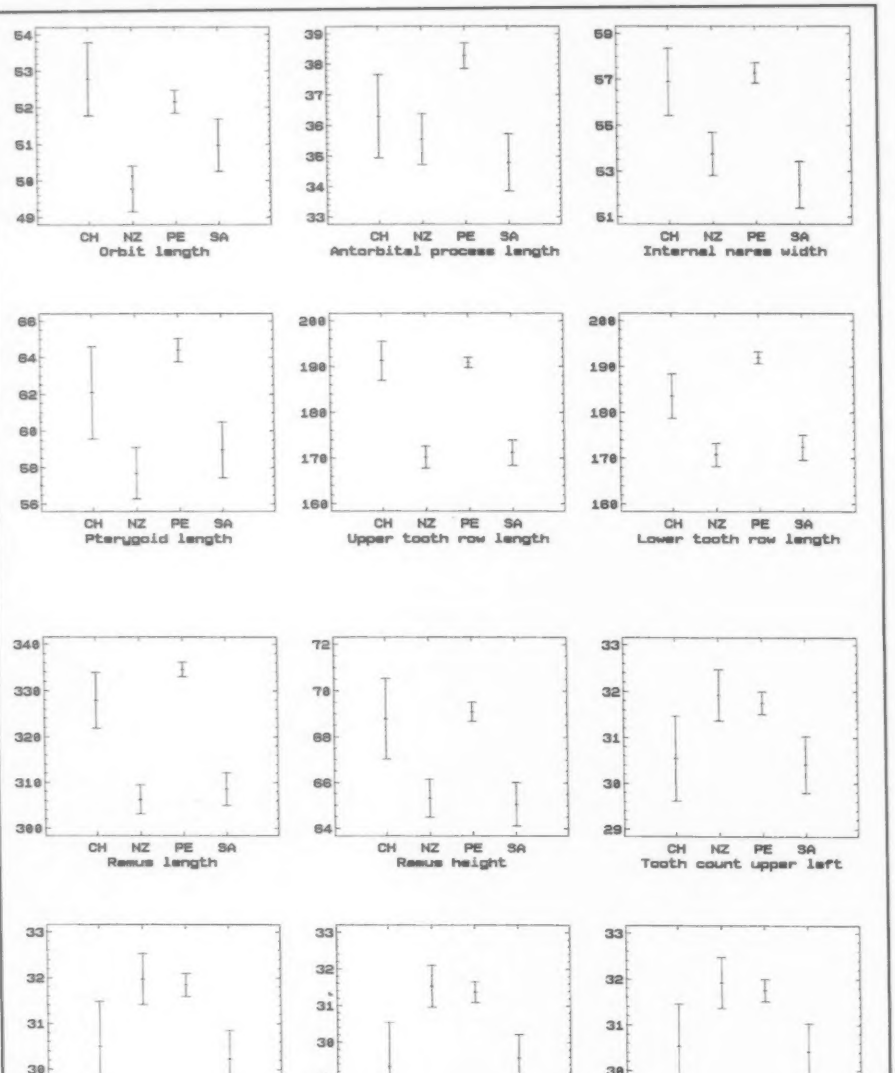

Figure 2 (Continued)

$P<0.0001$), presence of an accessory foramen above the foramen magnum (variable 16, $P=0.02$), and the degree of contact, posteriorly, between pterygoid hamuli (variable 23, $P=0.03$).

Upon comparison of frequencies for variables 16 and 23, two similarity pairs became apparent: New Zealand/southwestern Africa and Peru/Chile. Within each subgroup the monomorphic status for both characters was confirmed (chi-square, df=1, $P>0.41$ in four tests). Southwest African *L. obscurus* differed very significantly in character 1R from all other stocks. By removing the African subsample, frequencies for 1R in the three remaining geographic units became homogeneous (chi-square=0.58; df=4, $P=0.96$).

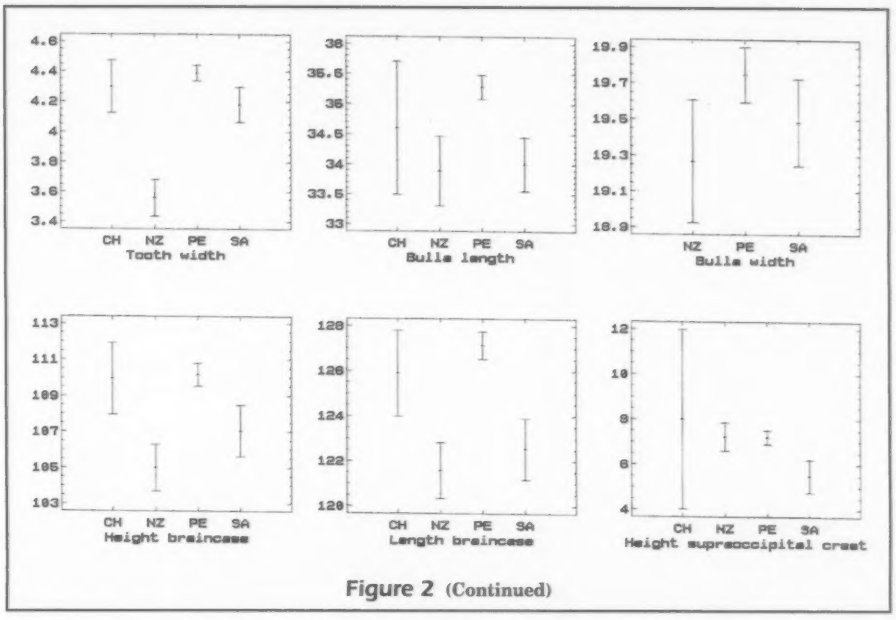

Figure 2 (Continued)

Discussion

Advanced fusion in the frontal-supraoccipital suture (FROC) is the most reliable single cranial criterion of sexual maturity in the dusky dolphin (95% efficiency). In combination with fusion=2 in LAC and ZYG cranial sutures, it can select sexually adult specimens from an undocumented series of *L. obscurus* skulls with even a higher degree of certainty. Distal fusion of premaxillary and maxillary bones has served as the indicator of choice for sexual maturity in *Stenella* spp. (Dailey and Perrin, 1973; Perrin, 1975a; Schnell et al., 1982[8]; Douglas et al., 1984, 1986; Perrin et al., 1981, 1987, 1989), common dolphin (Evans, 1975, 1982), and bottlenose dolphin (Walker, 1981[7]; Mead and Potter, 1990; Van Waerebeek et al., 1990). Its reliability and usefulness however depends on the species and the requirements of selection; Mead and Potter (1990) for instance reported that some immature *Tursiops* skulls were erroneously assigned to the adult group. Almost half of sexually mature dusky dolphins did not show any rostral distal fusion. The character reportedly was of no use in physically mature Pacific white-sided dolphins *Lagenorhynchus obliquidens* (Walker et al., 1986). Classification of specimens with heavily eroded sutures from weathering, boiling or chemical treatment is difficult, and if available sample size permits, these skulls should probably not be used. Moderate heating applied over short periods, to soften tissues for instance, inflicts little damage.

Skulls of *L. obscurus* exhibit little sexual dimorphism, therefore it was not possible to define usable classification functions for sexing specimens. Of 28 non-metrical cranial characters, none exhibited dimorphism. In addition, the number of dimorphic metrical characters (6 of 37, 16.2%) was remarkably low in comparison with that in other small delphinids such as the pantropical spotted dolphin *Stenella attenuata* (23 of 36, 63.9%; Schnell et al., 1985) and the spinner dolphin *Stenella longirostris* (13 of 36, 36.1%; Douglas et al., 1986). *L. obscurus* and the named *Stenella* spp. share the characteristic of males having a broader rostrum and a longer temporal fossa than females, while the overall skull size is not different between sexes (see Schnell et al., 1985; Douglas et al., 1986). Intersexual differences in these species may be governed, at least partly, by similar selection factors. A number of authors have invoked common (genetic) ancestry between *L. obscurus* and the *Stenella* species group to explain certain morphological similarities (True, 1889; Fraser, 1966; Fraser and Purves, 1960; Mitchell, 1970). However, I concur with the opinion of W. F. Perrin[13] that con-

[13]W. F. Perrin, Natl. Mar. Fish. Serv. Pers. commun. March 1992.

Table 6

Geographic variation in skull measurements and meristics of *Lagenorhynchus obscurus* from Peru (PE, *N*=189), Chile (CH, *N*=22), New Zealand (NZ, *N*=47) and southwestern Africa (SA, *N*=40).

	PE versus CH		NZ versus SA	
Variable	*t*	*P*	*t*	*P*
CBL	–0.48	0.40	0.48	0.63
RL	0.03	0.98	–0.26	0.80
RWB	–0.67	0.50	–0.45	0.65
RTEN	–1.12	0.27	1.6	0.11
RTIN	–0.34	0.73	1.32	0.19
PROW	2.50	<0.05	–0.094	0.93
POOW	–0.41	0.68	0.29	0.77
ZYW	–0.53	0.60	–0.074	0.94
PARW	0.84	0.40	–0.52	0.60
GWPMX	0.69	0.49	–0.14	0.89
ENW	–1.33	0.19	–2.02	<0.05
TFW	–0.11	0.91	–2.18	<0.05
ORL	1.03	0.30	–2.38	<0.05
APL	–2.89	<0.005	1.11	0.27
INW	–1.07	0.29	2.32	<0.05
PTEL	–2.23	<0.05	–1.28	0.21
UTRL	–0.17	0.87	–0.56	0.58
LTRL	–3.29	<0.005	–0.86	0.39
RAL	–2.10	<0.05	–0.97	0.36
RAH	–0.50	0.62	0.41	0.68
TCUL	–2.71	<0.01	3.45	<0.001
UR	–2.70	<0.01	4.25	<0.0001
TCLL	–3.01	<0.005	4.24	<0.0001
TCLR	–2.76	<0.01	3.95	<0.0002
TW	–1.05	0.30	–7.14	<0.0001
BL	–1.32	0.19	0.33	0.74
BW	—	—	–1.12	0.27
HBR	0.09	0.93	–2.09	<0.05
LBR	–1.37	0.17	–1.02	0.31
PEL	0.52	0.61	1.0	0.33
HCR	—	—	3.43	<0.005

vergence or parallellism is a more likely cause of the cited resemblances. The near absence of sexual dimorphism in the skull and external features of the dusky dolphin, coupled to the exceptionally large relative testis size, led me to suggest that sperm competition should occur in this species (Van Waerebeek, 1992b).

Both bivariate and multivariate analysis provide evidence of extensive geographic variation in skull morphology among the dusky dolphin stocks studied. Most strikingly, adult skulls from the Southeast Pacific stock are considerably larger than either New Zealand or SW African specimens (difference in weighted CBL means is 31 mm or 8.5% of CBL); skulls from New Zealand do not differ significantly in size from those of SW Africa. This is corroborated by the greater mean body length (roughly 10 cm) at sexual maturity and asymptotic length in Peruvian dolphins than in SW African and New Zealand specimens (Van Waerebeek, 1992b). Two non-metrical cranial characters also support the existence of the Peru/Chile and New Zealand/

Table 7

Statistics for discriminant analysis utilizing 21 cranial characters of adult *Lagenorhynchus obscurus* skulls from Chile (CH, *N*=7), New Zealand (NZ, *N*=24), southwestern Africa (SA, *N*=22) and Peru (PE, *N*=135). Group numbers (under centroids) refer to these used in the discriminant function plot (Fig.3). SEP= Southeast Pacific (Chile and Peru combined).

Discriminant function	Eigenvalue	Relative percentage	Canonical correlation
1	2.202	76.02	0.829
2	0.523	18.06	0.586
3	0.171	5.91	0.382

Unstandardized discriminant function coefficients

	1	2	3
CBL	0.0096	–0.0466	–0.0730
RL	0.0134	0.0025	0.0672
RWB	0.0038	–0.0618	–0.0057
RTEN	–0.0074	–0.0209	0.0268
PROW	–0.0285	–0.0416	–0.0647
POOW	0.0191	0.0528	–0.1291
ZYW	0.0152	–0.0285	0.1545
GWPMX	–0.0204	0.0431	0.0021
ENW	–0.0031	0.1414	–0.0817
TFW	0.0635	0.0256	–0.0028
ORL	–0.0190	0.0361	0.1625
APL	0.0683	–0.0198	–0.0739
INW	0.0481	–0.1066	0.0586
UTRL	0.0209	–0.0352	0.1097
LTRL	0.0664	0.0670	–0.1218
RAL	0.0067	0.0118	0.0047
TCUL	0.0195	–0.0537	–0.0424
TCUR	–0.1049	–0.1707	–0.1628
TW	0.6215	1.7912	0.6270
HBR	–0.0044	0.0669	–0.0467
LBR	0.0276	0.0058	0.0239
CONSTANT	–33.9754	5.8979	15.3204

Classification results

	Predicted group (percentage)				
Actual group	CH	NZ	SA	PE	SEP
CH	85.7	0	0	14.3	100
NZ	0	91.7	8.33	0	0
SA	0	4.6	90.9	4.6	4.6
PE	10.4	1.5	2.2	85.9	96.3

Group centroids

	Discriminant functions		
	1	2	3
CH (=1)	0.398	–0.705	2.040
NZ (=2)	–2.773	–1.254	–0.182
SA (=3)	–2.345	1.582	0.133
PE (=4)	0.855	0.0016	–0.095

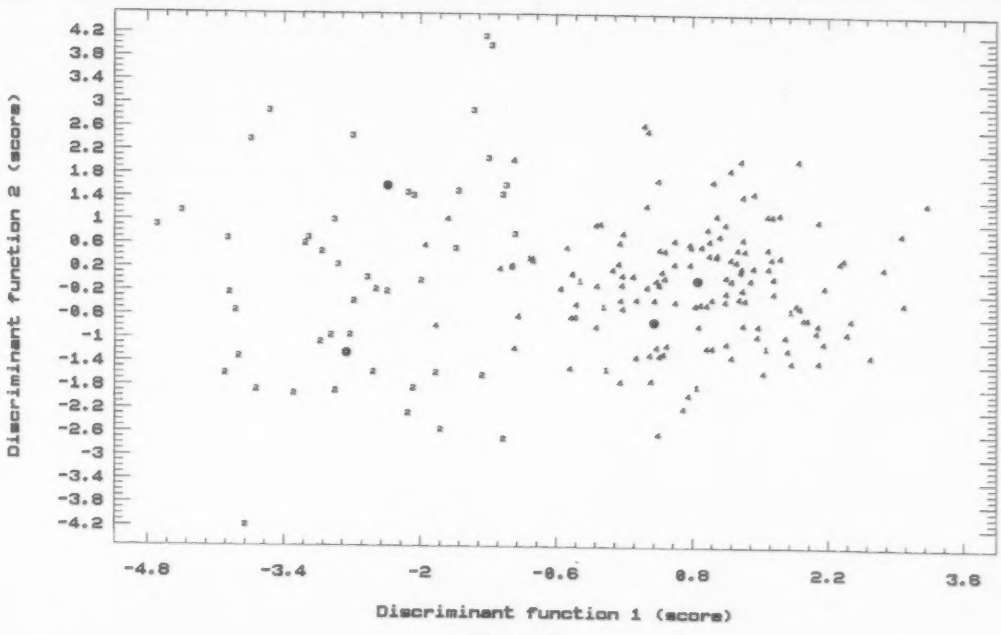

Figure 3

Scores of discriminant functions I and II based on 21 cranial characters for adult skulls of *Lagenorhynchus obscurus* from Chile (1), New Zealand (2), southwestern Africa (3), and Peru (4). Group centroids are indicated by a black dot. Corresponding statistics are presented in Table 7.

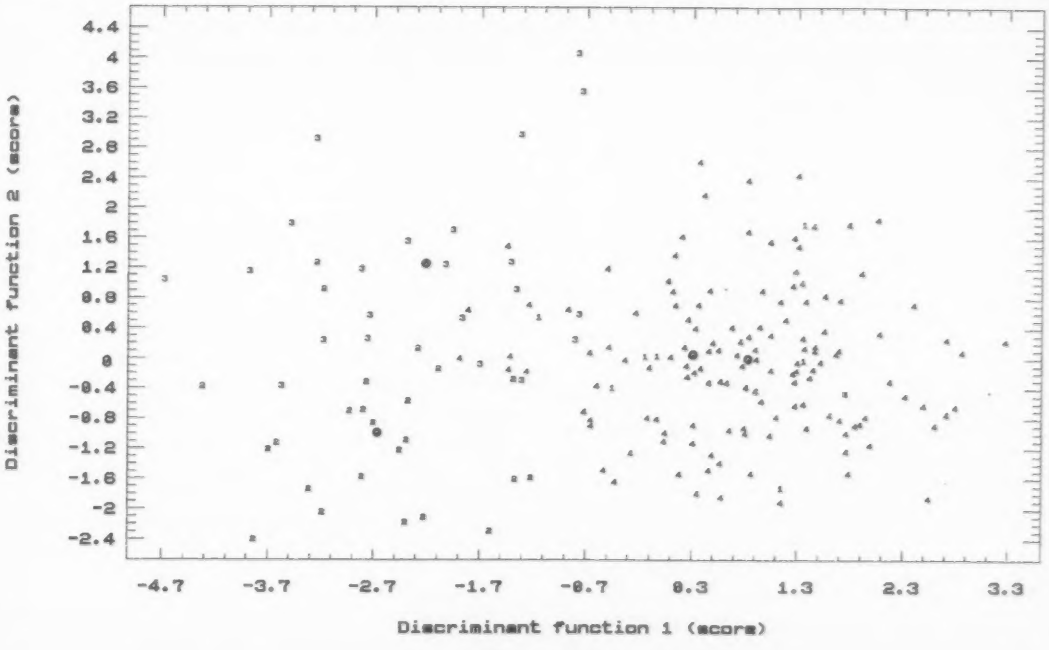

Figure 4

Scores of discriminant functions I and II based on the six most discriminating cranial characters (CBL, PROW, TFW, LTRL, TW, and TCUR) for adult skulls of *Lagenorhynchus obscurus* from Chile (1), New Zealand (2), southwestern Africa (3), Peru (4). Group centroids are indicated by a black dot. Corresponding statistics are presented in Table 8.

Table 8

Statistics of discriminant analysis utilizing a minimum number (6) of sexually monomorphic cranial variables with maximum discriminating power. Samples include adult skulls of *Lagenorhynchus obscurus* from Chile (CH, *N*=7), New Zealand (NZ, *N* =24), southwestern Africa (SA, *N*=22) and Peru (PE, *N*=135).

Discriminant function	Eigenvalue	Relative percentage	Canonical correlation
1	2.0188	84.35	0.818
2	0.3096	12.94	0.486
3	0.0649	2.71	0.247

Unstandardized discriminant function coefficients

	1	2	3
CBL	0.0350	–0.0588	–0.1105
PROW	0.0353	–0.0363	0.1205
TFW	0.0650	0.0484	0.0601
LTRL	0.0663	0.0449	0.1227
TW	0.7749	2.0950	–0.5369
TCUR	–0.0582	–0.3046	0.1422
CONSTANT	–36.6466	18.7270	–5.0552

Classification results

	Predicted group (percentage)				
Actual group	CH	NZ	SA	PE	SEP
CH	71.4	0	0	28.6	100
NZ	0	87.5	12.5	0	0
SA	9.1	18.2	72.7	0	9.1
PE	19.3	2.2	3.7	74.8	94.6

Group centroids

	Discriminant functions		
	1	2	3
CH (1)	0.301	0.0355	–1.281
NZ (2)	–2.674	–0.986	0.0236
SA (3)	–2.218	1.235	0.0400
PE (4)	0.821	–0.0278	0.0557

SW Africa similarity pairs. The New Zealand form is set apart from all others by minimal tooth size and is further distinguished from the SW African form by the significantly higher number of teeth.

A commonly used criterion of subspecific level difference between populations is whether or not 90% of the specimens can be unequivocally assigned to the proper group (Mayr, 1970). Perrin (1975a, 1990), for instance, described three subspecies of spinner dolphin, *Stenella longirostris,* based on this principle. Discriminant analysis allows correct identification of a group (96.5%, 91.7%, and 90.9%) of *L. obscurus* skulls from respectively the Southeast Pacific, New Zealand, and SW Africa, which warrants allocation of subspecific status. It seems premature, however, to formally describe subspecies and assign trinomial names, because no skulls from Argentina have been studied and the Chilean sample was too small to permit definitive conclusions on the degree of heterogeneity in the Southeast Pacific group. In particular, no skulls have been examined from dusky dolphins occurring off southern Chile (south of 46°S), which are thought to belong to the Argentinian stock (Van Waerebeek, 1992a). Considering the persisting mortality levels in gillnet and harpoon fisheries off Peru and northern Chile and in purse-seine operations off Argentina (Van Waerebeek and Guerra, 1986[5]; Guerra et al. 1987; Crespo and Corcuera, 1990[14]; Van Waerebeek and Reyes, 1993; Van Waerebeek, unpubl. data), the question of separate subspecies or population status is of major importance with respect to conservation.

The MANOVA results, suggesting some seasonal morphological variation in *L. obscurus* caught off central Peru, were not confirmed by other tests. However, the picture may be blurred by multi-year pooling or other sampling bias. High-resolution molecular techniques, such as DNA-fingerprinting, should preferably be used to explore this issue.

Although results were complementary, the resolving power of non-metrical cranial characters (not including tooth counts) was inferior to that of measurements (i.e., fewer characters differed); therefore non-metrical characters are not recommended as sole method for subspecific discrimination. Based on studies of the common dolphin, *Delphinus delphis*, Perrin et al. (1989) independently concluded that non-metrical characters be used only in combination with metrical characters and that special attention be paid to controlling conditions to maximize comparability of data sets.

Although little information is available on parasite prevalences in dusky dolphins other than these from Peru, preliminary indications of differential infestations add weight to the morphological findings. Crassicaudid nematodes were practically absent in the cranial sinuses of many hundreds of Peruvian dusky dolphins but were retrieved from the cranial sinuses of one out of two specimens from SW Africa.

[14]Crespo, E. A., and J. F. Corcuera. 1990. Interactions between marine mammals and fisheries in some fishing areas of the coast of Argentina and Uruguay. Doc. SC/090/G2 presented to the IWC, La Jolla, CA, 20–21 October 1990.

Table 9

Frequency distributions for selected non-metrical character states (coded 1–6, see Perrin et al., 1982) in 173 mature (MAT) and 48 immature (IMM) skulls of *L. obscurus* (sexes pooled) from central Peru. Chi-square contingency analyses indicate levels of developmental variation. For df=1, a Yates' corrected chi-square was computed and the higher of the two values (lower *P*) tabulated. Characters are explained in Table 1.

		Non-metrical character states								
		1	2	3	4	5	6	χ^2	*P*	df
1L	MAT	72	61	40				8.5	0.014	2
	IMM	14	28	6						
1R	MAT	56	17	99				4.89	0.09	2
	IMM	22	7	19						
2L	MAT	5	167	1				1.71	0.43	2
	IMM	0	48	0						
2R	MAT	9	162	2				3.21	0.20	2
	IMM	0	48	0						
3	MAT	45	83	45				5.36	0.07	2
	IMM	6	23	19						
5L	MAT	5					166	0.09	0.77	1
	IMM	1					46			
5R	MAT	2	1				168	0.52	0.77	2
	IMM	1	0				46			
6L	MAT	147	22	3			1	1.14	0.77	3
	IMM	42	6	0			0			
6R	MAT	162	10				1	1.38	0.50	2
	IMM	47	1				0			
9L	MAT	2	171					0.56	0.45	1
	IMM	0	48							
9R	MAT	6	166					1.72	0.19	1
	IMM	0	48							
10	MAT		172					—	—	—
	IMM		48							
12L	MAT	173								
12R	MAT	172						—	—	—
	IMM	48								
13	MAT	1		165	1			0.58	0.75	2
	IMM	0		48	0					
16	MAT	3	170				0.84	0.36	1	
17	MAT	77	96					8.83	0.003	1
	IMM	33	15							
18L	MAT	35	2				133	7.3	0.026	2
	IMM	8	4				36			
18R	MAT	41	3				126	0.97	0.61	2
	IMM	11	2				35			
19	MAT		2				169	0.57	0.45	1
	IMM		0				48			
23	MAT	149	5					1.36	0.24	1
	IMM	38	3							
24	MAT	123	34					3.15	0.08	1
	IMM	38	4							
25	MAT	118	38					5.97	0.01	1
	IMM	39	3							
30	MAT	9	158	0				6.20	0.04	2
	IMM	0	43	1						
33L	MAT		7	48	60	22	3	11.2	0.02	4
	IMM		0	24	12	2	1			
33R	MAT	1	12	52	52	18	4	4.57	0.47	5
	IMM	0	1	17	13	8	0			
34L	MAT	115	23	1				1.25	0.53	2
	IMM	35	4	0						
34R	MAT	118	21	0				1.43	0.23	1
	IMM	36	3	0						

Table 10

Geographic variation in non–metrical cranial (NMC) characters for 395 skulls of *Lagenorhynchus obscurus* from Peru (PE, N =221), Chile (CH, N =40), New Zealand (NZ, N =69), and southwestern Africa (SA, N =65). Frequency distributions of NMC states (coded 1–6, see Perrin et al. 1982) for each region and results of chi–square contingency analyses are presented. Samples include all sex/maturity groups except neonates; characters known to be maturity dependent (see Table 9) have been excluded.

NMC	REG	1	2	3	4	5	6	χ^2	P	df
1R	PE	78	24	118				31.8	<0.0001	6
	CH	10	4	16						
	NZ	21	6	37						
	SA	24	22	15						
2L	PE	5	215	1				2.8	0.83	6
	CH	0	30	0						
	NZ	1	62	0						
	SA	0	61	0						
2R	PE	9	210	2				5.9	0.43	6
	CH	0	30	0						
	NZ	1	62	0						
	SA	0	61	0						
3	PE	51	106	64				5.9	0.43	6
	CH	7	17	6						
	NZ	17	33	13						
	SA	11	27	23						
5L	PE	6	0				212	8.33	0.21	6
	CH	1	0				26			
	NZ	0	0				62			
	SA	3	1				55			
5R	PE	3	1				214	10.8	0.10	6
	CH	1	1				25			
	NZ	0	0				62			
	SA	3	0				57			
6L	PE	189	28	3			1	7.9	0.54	9
	CH	23	5	0			1			
	NZ	51	12	0			0			
	SA	49	10	0			1			
6R	PE	209	11				1	0.89	0.99	6
	CH	27	2				0			
	NZ	59	3				0			
	SA	57	3				0			
9L	PE	2	219					3.85	0.28	3
	CH	1	26							
	NZ	0	63							
	SA	0	60							
9R	PE	6	214					4.94	0.18	3
	CH	3	24							
	NZ	2	60							
	SA	3	57							
10	PE		220					—	—	—
	CH		28							
	NZ		64							
	SA		60							
12L	PE	221	0					7.38	0.06	3
	CH	27	0							
	NZ	61	1							
	SA	57	2							
12R	PE	220	0					7.38	0.06	3
	CH	28	0							
	NZ	62	1							
	SA	57	2							

Table 10 (Continued)

NMC	REG	1	2	3	4	5	6	χ^2	*P*	df
13	PE	1		213	1			2.41	0.88	6
	CH	0		25	0					
	NZ	1		62	0					
	SA	0		59	0					
16	PE	3	218					9.59	0.02	3
	CH	0	21							
	NZ	3	59							
	SA	5	53							
18R	PE	52	5	0			161	7.06	0.32	6
	CH	2	1	0			15			
	NZ	12	0	0			47			
	SA	7	0	1			48			
19	PE	0	2				217	6.48	0.37	6
	CH	0	0				16			
	NZ	0	0				60			
	SA	1	0				55			
23	PE	187	8					8.83	0.03	3
	CH	12	1							
	NZ	41	6							
	SA	41	7							
24	PE	161	38					3.8	0.28	3
	CH	12	2							
	NZ	34	13							
	SA	34	14							
33R	PE	1	13	69	65	26	4	7.24	0.95	15
	CH	0	0	7	4	1	0			
	NZ	0	5	16	16	5	1			
	SA	0	5	24	18	3	1			
34L	PE	150	27	1				8.59	0.20	6
	CH	5	5	0						
	NZ	36	8	0						
	SA	41	9	0						
34R	PE	154	24	0				2.41	0.49	3
	CH	10	3	0						
	NZ	36	8	0						
	SA	47	5	0						

Cyamid amphipods have never been found on *L. obscurus* from Peru (Van Waerebeek, 1992b), whereas in New Zealand they seem to be fairly common (Cipriano, 1985).

The actual distribution of *L. obscurus* populations is generally comparable to that of the four species of *Cephalorhynchus*, and the geographical dispersion may have similarly evolved under the influence of the eastflowing Westwind Drift, as proposed for *Cephalorhynchus* spp. by van Bree (1986) and Robineau (1989). The close affinity of the large-bodied Peruvian dusky dolphin with the Pacific white-sided dolphin (Webber, 1987; Van Waerebeek, 1992b) suggests that the former probably represents the most ancestral (plesiomorph) form of dusky dolphin from which the Argentinian, and small-bodied SW African and New Zealand forms were derived.

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Estimation of historical population size of the eastern spinner dolphin *(Stenella longirostris orientalis)*

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Abstract.—An assessment of the population status of the eastern spinner dolphin (*Stenella longirostris orientalis*) in the eastern tropical Pacific is required by the U.S. Marine Mammal Protection Act (MMPA), because dolphin are killed in the tuna purse-seine fishery. A pooled estimate of abundance from recent (1986–90) research vessel surveys, in combination with estimates of fisheries kills from tuna vessel observer data, was used to estimate the historical (pre-exploitation) population size with a population dynamics model. Estimates of relative population size (current population size divided by historical population size) were calculated by using a range of values for the maximum net recruitment rate and the maximum net productivity level (MNPL). The resulting estimates of relative population size ranged from 0.32 to 0.58, with a best estimate of 0.44 based on available life history data. Estimates of relative population size were all below the value of MNPL used to calculate each estimate. Calculation of confidence limits for relative population size by Monte Carlo simulation showed that the precision of the estimates was sufficient to make a status determination. The results indicated that, as of 1988, the stock of eastern spinner dolphin was depleted as defined by the U.S. MMPA.

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Fishery Bulletin 91:775–787 (1993).

The range of the eastern spinner dolphin, *Stenella longirostris orientalis* (Perrin, 1990), is entirely contained within the eastern tropical Pacific (Fig. 1). An assessment of population condition or status of this stock is required under the U.S. Marine Mammal Protection Act (MMPA), because eastern spinner dolphins are killed in the tuna purse-seine fishery, which includes some U.S. vessels, that occurs in this region. The MMPA requires that each marine mammal population be maintained at an "optimum sustainable population" (OSP) level, which has been defined by the U.S. National Marine Fisheries Service as a population size between the maximum net productivity level (MNPL) and carrying capacity (Federal Register, 21 December 1976, 41FR55536). Therefore, assessing the status of a marine mammal stock involves, if possible, determining if it is above its MNPL. Populations shown to be below MNPL are considered depleted under the MMPA.

One method for determining a population's status relative to MNPL is to estimate historical abundance, meaning abundance prior to significant fisheries mortality, which is assumed equivalent to the equilibrium population size (i.e., carrying capacity). The current population size is then compared with what is thought to be the MNPL for the population, given the estimate of equilibrium population size (Gerrodette and DeMaster, 1990). The historical abundance of several cetacean populations has been estimated by back-calculating from a current abundance estimate, with a population model and annual records of the number of animals harvested (Reilly, 1981; Breiwick et al., 1980, 1984; Lankester and Beddington, 1986). Smith (1983) described a method for back-calculating historical population size (N_h) for spinner and spotted dolphins (*Stenella* spp.) from estimates of the current population size (N_c), the historical kill in the tuna fishery, the maximum net recruitment rate (R_m), and the maximum net productivity level. He used this technique to estimate historical abundance for the eastern spinner dolphin, resulting in estimates of relative population size (N_c/N_h) for 1979 ranging from 0.17 to 0.25.

An estimate of N_h for a population of spinner or spotted dolphins, which have a relatively low R_m, can be very sensitive to the estimate of N_c, as long as the time period between N_c and N_h is not too great (Smith and Polacheck, 1979). Over a long time period (138 years), the estimate of N_h has been shown to be insensitive to the estimate of N_c for a baleen whale (*Balaena mysticetus*) population with a similarly low R_m (Breiwick and Braham, 1990). However, for a population that has experienced a relatively recent decline from known losses, the estimate of N_h should still be sensitive to the estimate of N_c (Gerrodette and DeMaster, 1990).

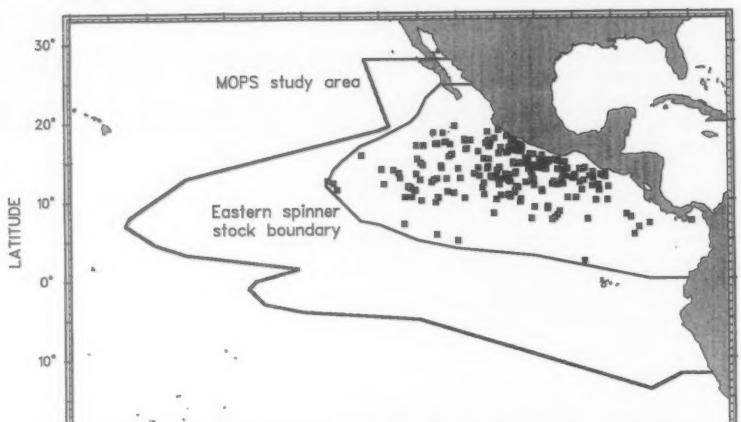

Figure 1

Distribution of the eastern spinner dolphin (*Stenella longirostris orientalis*) in the eastern tropical Pacific. Squares represent positions of all sightings from the 1986–90 Monitoring of Porpoise Stocks (MOPS) surveys used in the abundance estimate (a total of 236 sightings). The outer grey line represents the MOPS study area, and the inner solid line represents the area occupied by the eastern spinner dolphin.

For N_c, Smith (1983) used an estimated abundance of 293,000 animals for the eastern spinner dolphin, which was based on combined data from aerial and research vessel surveys conducted in 1979 (Holt and Powers, 1982). Recently, the U.S. National Marine Fisheries Service conducted large-scale research vessel surveys annually for five years (1986–90) as part of the Monitoring of Porpoise Stocks (MOPS) program, resulting in a revised estimate of abundance of 632,700 (Wade and Gerrodette, 1992b[1]). For a number of reasons, discussed below, this estimate should be more reliable (both more precise and less biased) than the 1979 estimate of abundance used by Smith (1983).

This revised abundance estimate was sufficiently different from the 1979 estimate to justify re-estimation of historical population size for the eastern spinner dolphin. Additionally, estimates of the historical kill have also been revised since Smith (1983), although they did not differ greatly from the previous estimates (Lo and Smith, 1986; Wahlen, 1986). Therefore, I estimated the historical population size for the eastern spinner dolphin using the same methods and the same ranges for the parameters R_m and MNPL as Smith (1983), but with revised abundance and fishery mortality estimates. This resulted in new estimates of relative population size for this stock.

Confidence limits for the estimates of relative population size were calculated by using Monte Carlo simulation methods (Buckland, 1984). These confidence limits only incorporated uncertainty due to sampling error of the current population estimate and the mortality estimates. They did not incorporate uncertainty in the model parameters R_m and MNPL. Therefore, confidence intervals were calculated for all parameter combinations.

Population abundance estimate

The MOPS cruises (1986–90) had approximately five times more kilometers of survey effort in the region occupied by eastern spinner dolphins than the 1979 survey. About 75% of the 1979 survey was concentrated within 1,000 km of the coast, whereas the range of the eastern spinner dolphin is up to 2,000 km from the coast (Fig. 1, Perrin et al., 1985). Therefore, the 1979 survey provided little coverage of the western half of the area occupied by eastern spinner dolphin (Holt and Powers, 1982, fig. 1). Raw sample sizes show the large difference in the quantity of data: a total of 285 schools containing eastern spinner dolphins were recorded during the MOPS surveys; a total of only 41 schools, during the 1979 survey. The large increase in

[1]Wade, P. R., and Gerrodette, T. 1992b. Estimates of cetacean abundance in the eastern tropical Pacific. Paper SC/44/018 presented at the annual meeting of the Int. Whal. Comm., June 1992.

the quantity of data made the MOPS estimates of abundance more precise for this stock, whereas the increased coverage of the stock range reduced the potential bias of geographical variation in abundance.

Both Holt and Powers (1982) and Wade and Gerrodette (1992b[1]) used line-transect analysis methods (Burnham et al., 1980) to estimate abundance. However, the relatively low number of sightings that resulted from the 1979 survey required an analysis technique that pooled sightings of different stocks and species of dolphin to estimate the abundance of each stock (Holt and Powers, 1982). Although the same technique was used initially to estimate annual abundance for the first four years of the MOPS data (Holt and Sexton, 1989, 1990, a and b; Gerrodette and Wade, 1991), the greater number of sightings in each year made this unnecessary. Therefore, to examine trends in abundance, a revised analysis of all five years of MOPS data was undertaken in which annual estimates of abundance for each stock were made only from sightings of that stock (Wade and Gerrodette, 1992a). These estimates were considered to be less biased estimates of abundance than earlier estimates available for eastern tropical Pacific dolphins (IWC, 1992). No significant trend in abundance for eastern spinner dolphins was observed over this short period, but the power of detecting a trend was low (Gerrodette, 1987; Wade and Gerrodette, 1992a). The five annual estimates of abundance for the eastern spinner dolphin ranged from 391,200 to 754,200, with a mean of 588,500.

Wade and Gerrodette (1992a) discussed in detail the differences between their analysis technique and the Holt and Powers (1982) technique, but I will briefly summarize the two major differences here. First, Holt and Powers (1982) calculated a single effective strip width (i.e., 2.0/f(0), Burnham et al., 1980) for all dolphin species, whereas Wade and Gerrodette (1992a) estimated a separate value for each stock. These effective strip widths varied substantially between the different dolphin stocks, ranging from a low of 2.5 km to a high of 11.9 km (Wade and Gerrodette, 1992a), indicating that the Holt and Powers (1982) technique may have introduced considerable bias by pooling across different stocks and species.

Second, Holt and Powers (1982) estimated the abundance of each stock by making a pooled estimate for each species, and then divided the species estimate between the stocks of that species according to the relative size of the area occupied by each stock. For example, an estimate of spinner dolphin abundance was made by pooling sightings of eastern spinner dolphins with sightings of whitebelly spinner dolphins, a different morphological form that is distributed farther offshore and partially overlaps the area occupied by the eastern spinner dolphin (Perrin et al., 1985, 1991). The abundance estimate for the eastern spinner dolphin was then made by multiplying this pooled estimate by the ratio of the area occupied by the eastern spinner dolphin to the sum of that area plus the area also occupied by the whitebelly spinner dolphin. This approach would only be un-biased if the two stocks had exactly the same density (number of animals per unit area) within their respective stock areas. There is no reason to assume this is true; therefore, an analysis based solely on sightings of eastern spinner dolphin, as in Wade and Gerrodette (1992a), is likely to be less biased.

To obtain a best estimate of absolute abundance, the five years of MOPS data were pooled across years for a second analysis to estimate average abundance for the period for 25 stocks of cetaceans in the ETP, including the eastern spinner dolphin (Wade and Gerrodette, 1992b[1]). The analysis technique of Wade and Gerrodette (1992a) was used, supplemented by a technique for prorating sightings from unidentified categories. Abundance estimate from this analysis should represent the (least biased and most precise) abundance estimate currently available for eastern spinner dolphin, and was therefore used as the starting point for the back-calculations. A summary of the methods and results from that paper for the eastern spinner dolphin has been presented here.

Methods

Pooled (1986–90) abundance estimate

The methods of Wade and Gerrodette (1992a) were mostly repeated but were applied to all five years of data together rather than separately to each year by itself. Population abundance (N) of eastern spinner dolphins was computed by line-transect methods (Burnham et al., 1980) as:

$$N = \sum_{k=1}^{4} N_k, \tag{1}$$

where

$$N_k = \frac{n_k f_k(0)}{2 L_k} S_k A_k \tag{2}$$

and

N_k = abundance estimate for eastern spinner dolphins in stratum k,
n_k = number of eastern spinner dolphin schools in stratum k,
$f_k(0)$ = detection function in stratum k, evaluated at zero distance,
S_k = mean school size for eastern spinner dolphin schools in stratum k,

L_k = total effort in stratum k in kilometers,
A_k = total area in stratum k in square kilometers.

This represents a stratified analysis, where only sightings from a stratum were used to calculate the density and, therefore, abundance within the stratum. Abundance estimates for each stratum were summed across the four strata to get a total estimate for the stock. The only change in methodology from Wade and Gerrodette (1992a) involved the calculation of f(0). In that analysis, f(0) was estimated by pooling across strata because of inadequate sample sizes in each stratum in each year. With the larger sample sizes available from pooling the five years of data, there were enough sightings in the inshore and middle strata (Wade and Gerrodette, 1992a; fig. 1) to estimate f(0) independently in each stratum. A third stratum (west) on the edge of the stock area had only four sightings, so a single pooled estimate of f(0) was estimated for the middle and west strata. As expected, because it was outside of the range of eastern spinner dolphin (Perrin et al., 1985), there were no sightings in the fourth stratum (south). A hazard rate model (Buckland, 1985) was fit to the data to estimate f(0). The perpendicular distances were truncated at 5.5 km, because not all dolphin schools further than 5.5 km perpendicular distance were pursued for species identification and school size estimation.

Eastern and whitebelly spinner dolphins partially overlap in range, but can be distinguished from each other by their color pattern and morphology (Perrin, 1990; Perrin et al., 1991). Out of 134 sightings of spinner dolphins in the area of overlap between the two stocks, 16 were, for various reasons, unidentified to stock. Those sightings were prorated to the eastern stock of spinner dolphin by using the estimated proportion of spinner dolphin in the overlap area from the eastern stock (Wade and Gerrodette, 1992b[1]). Similarly, sightings of unidentified dolphins were prorated to the eastern stock, based on the estimated proportion of dolphins from the eastern stock in each stratum (Wade and Gerrodette, 1992b[1]). The prorated portions of unidentified spinner dolphin and unidentified dolphin were added to the original estimate to give a final estimate of abundance. The standard error of the abundance estimate was calculated by bootstrap methods (Efron, 1982), by using legs of effort as the resampling unit, with 1,000 iterations.

Fisheries kill estimates

Estimates of dolphin kill from the tuna fishery in the ETP have been revised since Smith (1983). Lo and Smith (1986) presented revised kill estimates for 1959–1972, and Wahlen (1986) presented revised kill estimates for 1973–1978, in each case with associated standard errors. Additionally, kill estimates for 1979–87, with associated standard errors, have been published (IATTC, 1989). However, Lo and Smith (1986) reported total dolphin kill and did not divide it into stock categories, while Wahlen (1986) reported kill estimates by stock, but only for the U. S. tuna vessel fleet. Therefore, I divided the estimates of Lo and Smith (1986) to stock by the same stock proportions used in Smith (1983). I adjusted the estimates of Wahlen (1986) using the estimated total number of sets, as reported in Punsly (1983). Wahlen (1986) reported the estimated number of sets by the U.S. fleet. I multiplied the kill estimate in each year from Wahlen (1986) by the ratio of the sets made by the entire fleet to the sets made by the U.S. fleet to produce an estimate of the total number of eastern spinner dolphins killed in each year. This assumes that the kill rates of the unobserved international fleet were the same as the U.S. fleet.

Population model

The methods of Smith (1983) were duplicated, by using the simple recursive relationship

$$N_{t+1} = N_t - K_t + R_t\,(N_t - \tfrac{1}{2}K_t), \tag{3}$$

where
N_t = population abundance in year t
K_t = fisheries kill in year t
R_t = net recruitment rate in year t.

Density-dependence is incorporated into the equation through the net recruitment rate, which is defined as

$$R_t = R_m\left[\,1 - \left(\frac{N_t}{N_h}\right)^z\,\right], \tag{4}$$

where
R_m = maximum net recruitment rate
z = shape parameter that sets the maximum net productivity level (MNPL)
N_h = historical population size (assumed to be the equilibrium population size).

For any value of R_m and MNPL, z can be calculated as in Polachek (1982). Equation 1 can be solved for N_t as a function of N_{t+1}, R_t, and K_t. Therefore, by specifying an initial population size, the number of animals killed in each year, the maximum net recruitment rate, and the maximum net productivity level, these two equations can be iteratively solved for N_h.

Estimates of R_m and MNPL

Values used by Smith (1983) for R_m were 0.0, 0.03, and 0.06, which he thought to encompass the range of possible values of R_m for spinner dolphins. No direct estimate of net reproductive rate (R) exists for eastern spinner dolphins because of the difficulty in estimating survival rates. The calving interval is approximately three years (Perrin and Reilly, 1984). The age of sexual maturity (ASM) has been reported as five years (Perrin and Henderson, 1984). However, a new study using a much larger data set estimated ASM for the eastern spinner dolphin to be approximately 10 years, by using data collected from 1974 to 1990[2]. This is similar to the estimate of approximately 11 years for the congener northern spotted dolphin, *Stenella attenuata* (Chivers and Myrick, 1991[3]; Myrick et al., 1986), which is found in the same region of the eastern tropical Pacific.

There are no estimates of survival rates for eastern spinner dolphin. Therefore, estimating the net reproductive rate for eastern spinner dolphin required using estimates of survival rates from another species. Among the best estimates of survival rates for a delphinid come from a long-term study of known individuals of a coastal population of *Tursiops truncatus*, with estimates of adult and calf survival of 0.96 and 0.80, respectively (Wells and Scott, 1992). From Reilly and Barlow (1986), those survival rates in combination with a calving interval of three years and an ASM of nine years resulted in an R of 0.03, which could be considered the best estimate of R for the eastern spinner dolphin. Those survival rates may be low, however, because the Wells and Scott (1992) study was of a population that was thought to be at equilibrium, as it had been relatively constant in abundance for many years. Using the maximum survival rates considered by Reilly and Barlow (1986) with the same calving interval (3 yr) and ASM (9 yr) results in an R of 0.05. If the eastern spinner dolphin was well below half its equilibrium population size in 1979 (Smith, 1983), then its net reproductive rate should have been very close to its maximum, R_m. For this paper I therefore considered 0.04 as the best estimate of R_m currently available for the eastern spinner dolphin, with 0.06 the greatest value of R_m possible. Therefore, the same range of values as in Smith (1983) was used for R_m, ranging from 0.00 to 0.06 by increments of 0.002, for a total of 31 values.

Values used by Smith (1983) for MNPL were 0.50, 0.65, and 0.80 (MNPL is expressed as a fraction of equilibrium population size in this paper), corresponding to z values (see Eq. 4) of 1.0, 3.482, and 11.216, respectively. These encompassed the range of actual values of MNPL for long-lived marine mammals, such as dolphins, based on work by Fowler (1981). No direct estimate of MNPL exists for the eastern spinner dolphin. Fowler (1984) gave evidence that MNPL was greater than 0.50 for cetaceans. A value of 0.60 is currently being used for management of cetaceans under the U.S. MMPA (Federal Register, 31 October, 1980, 45FR64548), and for this paper, will be considered the best working value of MNPL currently available for the eastern spinner dolphin. Values of z were used so that MNPL ranged from 0.50 to 0.80 (the same range as in Smith, 1983), by using increments of 0.01, for a total of 31 values. The exact value of z necessary to give the specified MNPL for any value of R_m was calculated as in Polachek (1982).

The 31 values used for both R_m and MNPL produced a total of 961 parameter combinations for which relative population size was estimated. This large number of parameter combinations allowed the calculation of contours for the estimate of relative population size as a function of the 2 parameters of the model.

Confidence limits for N_h

For every combination of the parameters R_m and MNPL, confidence limits for relative population size were calculated by a Monte Carlo simulation (Buckland, 1984) which incorporated the sampling error of the current abundance and kill estimates. On each of 1,000 iterations, an artificial data set was randomly generated by sampling values for the current abundance and for the fisheries kill in each year. These values were each drawn from Gaussian distributions with means and variances equal to the appropriate point estimates. Relative population size was then estimated for each of these artificial data sets, and 95% confidence limits for relative population size were calculated using the percentile method (Efron, 1982).

The kill estimates for 1959–1972 were not independent from each other, as Lo and Smith (1986) estimated the kill in each year by multiplying an average mortality-per-set for 1959–1972 by the number of fishing sets in each year. Therefore, on each simulation iteration the kill values for 1959–1972 were randomly generated with the same random deviate. This resulted in the kill values for those years being perfectly correlated amongst themselves from simulation

[2]Susan Chivers, Southwest Fish. Sci. Cent., La Jolla, CA. Pers. commun.

[3]Chivers, S. J., and A. C. Jr. Myrick. 1991. Comparison of age at sexual maturity for two stocks of offshore spotted dolphins subjected to different rates of exploitation. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Southwest Fish. Sci. Cent., P.O. Box 271, La Jolla, CA 92038. Admin. Rep. LJ-91-31, 19 p.

trial to trial, which correctly reflected the lack of independence in the actual estimates. The kill values for all other years were sampled independently.

Results

Estimates of abundance and kill

The five years of the MOPS surveys resulted in 236 sightings of eastern spinner dolphins used in the abundance estimate. The abundance estimate based solely on these sightings was 568,100. Adding prorated numbers of unidentified spinner and unidentified dolphin sightings resulted in a final estimate of 632,700, with a CV of 0.167 (Table 1). The fisheries kill estimates ranged from a high in 1961 of 138,000 to a low in 1983 of 700 (Table 2).

Contours of relative population size (N_c/N_h) as a function of R_m and MNPL ranged from 0.35 to 0.55 (Fig. 2). Relative population size increased with both R_m (growth rate) and MNPL (the amount of non-linearity in the density-dependence response). The lowest relative population size was 0.32, for the case of R_m=0.00, (i.e., no net growth in the population before fisheries kill was included). The highest relative population size was 0.58 for the case of the highest growth rate and MNPL (0.06 and 0.80, respectively). These low and high estimates of relative population size correspond to estimates of pre-exploitation abundance of 1,956,000 and 1,100,000, respectively. Relative population size increased by approximately 0.03 for every increase of 0.01 in R_m. The influence of MNPL was greater at higher growth rates, as relative population size increased by approximately 0.02 for every increase of 0.10 in MNPL at R_m=0.02, but increased by approximately 0.05 for every increase of 0.10 in MNPL at R_m=0.06. There were no combinations of parameter values such that relative population size was estimated to be above MNPL.

The upper 95% confidence limit for relative population size as a function of R_m and MNPL, based on the sampling error of the abundance and kill estimates, ranged from 0.45 to 0.91 (Fig. 3). The upper confidence limit was always above MNPL when R_m was greater than 0.046 (Fig. 3, shaded region). The lower 95% confidence limit for relative population size as a function of R_m and MNPL, ranged from 0.22 to 0.36 (Fig. 4).

All population trajectories declined until 1973 (Fig. 5), after which the estimated fisheries kill declined substantially (Table 2). For the highest growth rate, the population trajectory showed an increasing trend from 1976 to 1988 (Fig. 5, line C), whereas for the lowest growth rate the model resulted in a relatively stable population level between 1976 and 1988 (Fig. 5, line A).

The confidence limits around relative population size broadened with increasing R_m. For example, for a MNPL of 0.60, the confidence limits ranged from 0.23 to 0.44 for R_m=0.00, whereas they ranged from 0.33 to 0.72 for R_m=0.06 (Fig. 6). As in Smith (1983), relative population size was a linear function of R_m.

Discussion

For all parameter values of R_m and MNPL equal to those in Smith (1983), estimates of relative population size were higher in this analysis. For example, for R_m=0.03 and MNPL=0.65, Smith (1983) reported a relative population size of 0.20 versus a result of 0.42

Table 1

Estimate of abundance (in thousands of animals) of the eastern spinner dolphin (*Stenella longirostris orientalis*) from the Monitoring of Porpoise Stocks surveys (1986–90). Strata are identified in Figure 1.

	Abundance estimates			
	Total	Inshore	Middle	West
From eastern spinner dolphin schools	568.0	364.8	160.2	43.1
Prorated from unidentified spinner dolphins	15.4	9.0	6.0	0.4
Prorated from unidentified dolphins	49.2	37.5	10.9	0.8
Final estimate	632.7			
Standard error	105.7			
Coefficient of variation	0.167			
Upper 95% confidence limit	778.9			
Lower 95% confidence limit	403.2			

Table 2

Estimates of fisheries kill in thousands by year for the eastern spinner dolphin (*Stenella longirostris orientalis*). CV is the coefficient of variation of the kill estimate. Sources for the estimates are 1) 1959–72 from Lo and Smith (1986), using the stock proportions of Smith (1983); 2) 1973–78 from Wahlen (1986), adjusted for number of sets of total fleet in Punsly (1983); 3) 1979–87 from IATTC (1989). See text for explanation.

Year	Mortality	CV
1959	14.3	0.32
1960	124.3	0.31
1961	138.8	0.28
1962	56.2	0.25
1963	62.4	0.22
1964	101.4	0.20
1965	119.6	0.20
1966	97.2	0.15
1967	66.8	0.16
1968	59.5	0.15
1969	106.0	0.15
1970	107.4	0.15
1971	58.4	0.17
1972	87.4	0.16
1973	18.4	0.16
1974	17.8	0.11
1975	17.1	0.11
1976	14.7	0.12
1977	1.8	0.12
1978	1.1	0.11
1979	1.5	0.24
1980	1.1	0.20
1981	2.3	0.28
1982	2.6	0.33
1983	0.7	0.38
1984	6.0	0.52
1985	8.9	0.16

here. The different results must be due to either the use of revised estimates of abundance and kill or the use of 1988 as a starting point rather than 1979; these were the only differences between the analyses. As will be shown, most of the difference resulted from the higher estimate of current population size, although the lower revised kill estimates also contributed to a higher estimate of relative population size. Repeating the back-calculation of Smith (1983) from 1979, but using revised population and kill estimates, resulted in nearly the same estimate of relative population size as reported here. For example, for R_m=0.03 and MNPL=0.65, back-calculating from 1979 as opposed to 1988 resulted in an estimate of relative population size of 0.41 versus 0.42, whereas Smith (1983) reported a value of 0.20. An inspection of the population trajectories (Fig. 5) confirms that the difference was not due to the different starting year, as the model trajectories, except at the highest growth rates, indicated little change in the population size between 1979 and 1988. This also agrees with the independent results of Buckland et al. (1992), which indicated little difference in relative population size between those two years. Therefore, the difference in the results reported here and those of Smith (1983) should not be interpreted as a recovery in the population between 1979 and 1988. These new, higher estimates of status should instead be interpreted as a revision of the estimate of relative population size, due mostly to the improved abundance estimate available from the MOPS surveys.

The new estimates of relative population size, although higher than Smith (1983), are still below MNPL for all parameter combinations. Because the parameter values used encompassed those values possible for a spinner dolphin (Reilly and Barlow, 1986), this result indicated that, as of 1988, the eastern spinner dolphin was still well below its 1959 population size. With R_m=0.04 and MNPL=0.60, the population was estimated to be at 44% of its historical size. Even with the maximum value of R_m of 0.06, the population in 1988 was estimated to be 43% (MNPL=0.50) to 58% (MNPL=0.80) of its size in 1959. However, careful consideration must be given to several issues before accepting these results as valid. These issues include the precision (reflecting the precision of the abundance and kill estimates) and potential biases (reflecting either biased abundance and kill estimates or mis-specification of the model) of the result, and the quality of pre-1972 fisheries kill data.

Precision

The precision of the estimates of relative population size was investigated by simulation to explore the uncertainty of the results due to sampling error, under the assumptions that the population model and parameter values were true. This addresses the question of how likely the estimates of relative population size were below MNPL if the true relative population size was above MNPL, solely because of variability associated with sampling the current abundance and fisheries kill estimates. The upper 95% confidence limit of relative population size was below MNPL for the majority of the parameter combinations, moving above MNPL only for values of R_m greater than 0.018 (Fig. 3). If MNPL was assumed to be 0.60, then the upper 95% confidence limit of relative population size was only above MNPL for values of R_m greater than 0.034 (Fig. 3). The upper confidence limit was always above MNPL if R_m was at least 0.046. Viewed in a hypothesis testing context, this result indicated that the null hypothesis that relative population size was greater than MNPL in 1988 could be rejected for most of the parameter combinations. Only at higher growth rates could this hypothesis not be rejected. From sampling error alone, it was equally possible that the population was actually worse off than estimated, as the lower 95% confidence limits go as low as 0.22, and were as low as 0.28 even at the highest growth rate of R_m=0.06.

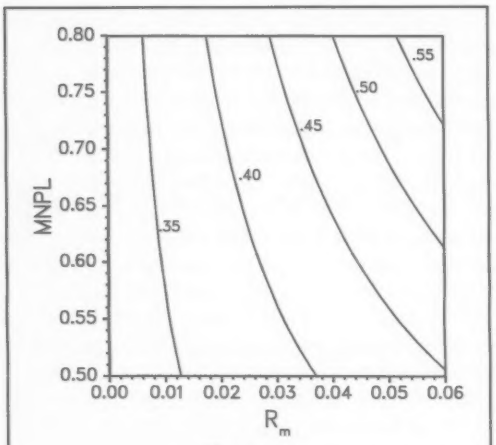

Figure 2

Contours of relative population size (current abundance divided by historical abundance) for the eastern spinner dolphin (*Stenella longirostris orientalis*), as a function of maximum net recruitment rate (R_m) and maximum net productivity level (MNPL).

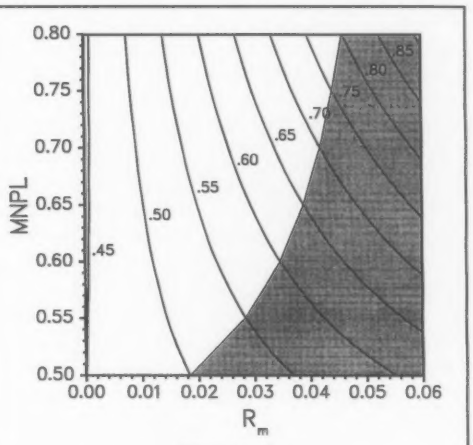

Figure 3

Contours of the upper 95% confidence limit for relative population size for the eastern spinner dolphin (*Stenella longirostris orientalis*), as a function of maximum net recruitment rate (R_m) and maximum net productivity level (MNPL). The shaded region represents the area where the confidence limit was above MNPL.

The confidence limits around relative population size were not much greater proportionally than the confidence limits around N_c (Fig. 6). For example, from the simulation the confidence limits for relative population size with values of 0.04 for R_m and 0.60 for MNPL were 0.29–0.62, representing a coefficient of variation (CV) of 19%, whereas the CV of N_c was 17% (Table 1). Although one might have expected the precision of the estimate of relative population size to be much less than the precision of N_c, this was not the case because of the independence of most of the kill estimates. Sampling variance in the kill estimates would therefore

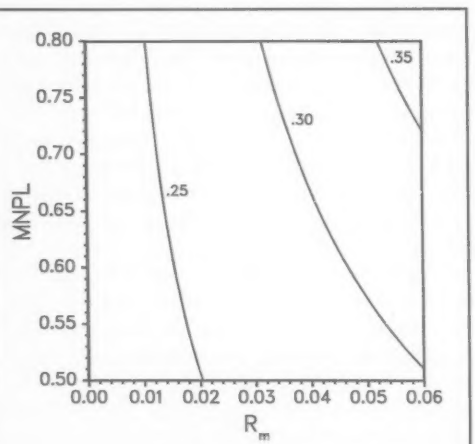

Figure 4

Contours of the lower 95% confidence limit for relative population size for the eastern spinner dolphin (*Stenella longirostris orientalis*), as a function of maximum net recruitment rate (R_m) and maximum net productivity level (MNPL).

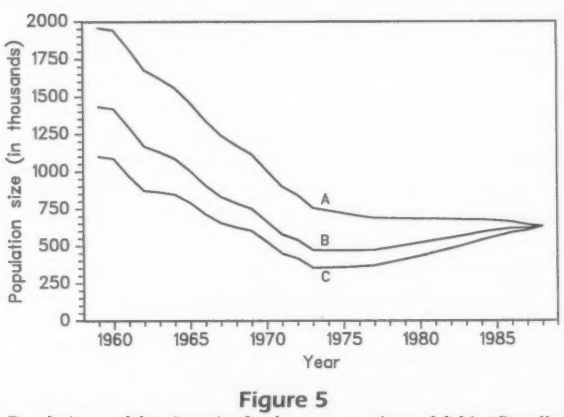

Figure 5

Population model trajectories for the eastern spinner dolphin (*Stenella longirostris orientalis*) for three different parameter combinations of maximum net recruitment rate (R_m) and maximum net productivity level (MNPL): (A) R_m=0.00 and MNPL=0.50, (B) R_m=0.04 and MNPL=0.60, and (C) R_m=0.06 and MNPL=0.80. A and C represent the lowest and highest estimates of relative population size, respectively. B represents the combination of the best estimates for the parameters based on available life history data.

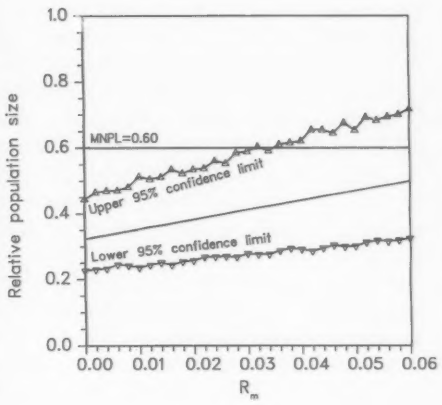

Figure 6

Point estimates and 95% confidence limits for relative population size for the eastern spinner dolphin (*Stenella longirostris orientalis*), as a function of the maximum net recruitment rate (R_m), for the estimate of the maximum net productivity level (MNPL=0.60) currently used for management under the U.S. Marine Mammal Protection Act.

tend to cancel itself, as over-estimates of kill in some years would be balanced by under-estimates in other years. However, systematic bias in the kill estimates would lead to a poor estimate of relative population size, creating a relatively precise yet inaccurate estimate. The same would be true for bias due to the use of an inappropriate model, or in the estimate of N_c. Potential biases in these three areas must therefore be considered.

Bias

Two major sources of bias may have existed in the fisheries kill estimates from 1972 to the present. One source of bias, that of observer effects, has been demonstrated and implies that less dolphin kill occurred on observed trips due to modifications in fishing behavior in response to the observer's presence (Wahlen and Smith, 1985). Unfortunately, there is no way to estimate the magnitude of the effect. The second potential source of bias was the lack of participation in data collection by some countries during some years, especially if significant differences in kill rates existed between countries. Most important may have been the lack of 1979–1986 data from Mexico (Edwards, 1989), a major component of the fishery whose kill rates may have been higher than average during that time period. These biases would lead to under-estimates of kill and thus over-estimates of relative population size.

Additional sources of bias existed in the pre-1972 kill estimates, because of both a lack of observations of mortality-per-set (MPS) in many years and because the MPS data prior to 1971 were not collected as part of a systematic observer program. Data on the number and types of sets were collected in every year, starting in 1959 (Punsly, 1983), but an observer program for collecting MPS data was not started until 1971 and random placement of observers until 1972 (Edwards, 1989). The moderate amount of MPS data collected in 1971 was potentially biased because most of the boats with observers were smaller and older, and may have had a higher MPS (Edwards, 1989; Lo and Smith, 1986, table 1). Most of the pre-1971 data were from scientists who were on the tuna boats for the purpose of collecting dolphin specimens, but who also recorded MPS data on their own initiative (Smith and Lo, 1983). There is no obvious reason why tuna vessels that agreed to allow scientists to collect specimens during their fishing operations would tend to have different mortality rates, but in a strict sense these were not random samples of fishing trips. Data from one fishing trip in 1964 were recorded and reported by a fisherman, who may have done so because of the magnitude of the kill, making those data potentially biased (Smith and Lo, 1983). However, the MPS data did not differ greatly in those years from the data collected in 1972 (Lo and Smith, 1986, table 1). Because of this and the greater quantity of MPS data available from 1972, estimates of 1959–1972 fisheries kill made by multiplying the average 1972 MPS rate by the number of sets in each year would not differ greatly from the estimates used here from Lo and Smith (1986), which were made by using the pooled 1964–1972 MPS rate.

Therefore, the fisheries kill would only have been over-estimated if the MPS in the pre-1971 unobserved years was lower than in 1972. However, MPS has consistently declined over time, declining most rapidly following the passage of the Marine Mammal Protection Act in 1972 (Smith 1983). No evidence exists that MPS could have been lower from 1959 to 1970 than it was in 1971–72. MPS may have been higher, especially before use of the back-down procedure had become widespread and well practiced (Perrin, 1969; Edwards, 1989). If it is assumed that MPS has only declined since the beginning of the fishery, the 1959–1970 kill estimates of Lo and Smith (1986) were likely under-estimates of the true kill. Thus, the major sources of bias in fisheries kill estimates all suggest that kill estimates were negatively biased.

Bias in the estimate of abundance could also bias the estimate of relative population size. Wade and Gerrodette

(1992a) discuss a number of sources of potential bias when applying line-transect theory to the MOPS survey data. Several potential sources of bias do not appear to have a major effect. Independent observer experiments indicate that few schools (and no large schools) were missed on the trackline (Wade and Gerrodette, 1992a). Aerial photographs have confirmed that little bias has been introduced by the observer's estimate of school size (Gerrodette and Perrin, 1991[4]). One partially unresolved issue is that of vessel avoidance by dolphin schools, which would bias the estimate downwards, although this may not have been a major problem (Au and Perryman, 1982; Hewitt, 1985). Additionally, mean school size is likely over-estimated owing to the decreased probability of detection of small schools at larger perpendicular distances (Drummer and McDonald, 1987). Although some stocks in the MOPS surveys appeared to be biased by as much as 20% by this problem, the eastern spinner and other stocks were not (Wade and Gerrodette, 1992a). Finally, the distribution of the eastern spinner dolphin is well known (Perrin et al., 1985) and is well within the MOPS study area (Fig. 1), so it can be concluded that the abundance estimate applies to the entire population. Therefore, the estimate of abundance did not contribute any major bias to the estimate of relative population size.

Bias may also have been introduced by assuming that the simple model specified in Equation 3 correctly models eastern spinner population dynamics, although a simulation study has shown that a simple model can perform as well as a more complex model for this type of analysis (Lankester and Cooke, 1987). The most important feature of eastern spinner population dynamics for this analysis is their inability to undergo large increases in population size from one year to the next. Their relatively low maximum population growth rate, which is due to the biological constraints of their life history discussed above, was incorporated into Equation 3 by using only biologically plausible values of R_m. The only way in which the actual population could have substantially differed from the model would be if the population had a much lower growth rate than expected in some years. For example, large interannual variations in oceanographic conditions related to El Niño events in the eastern tropical Pacific (Fiedler et al., 1992) may lead to large changes in the quantity of prey available for the dolphins. This could lead to lower growth rates in some years, which would cause the specified model to over-estimate relative population size.

Of more concern is the lack of age-structure in the model (Goodman, 1984[5]). The age-distribution of the northern spotted dolphin (*S. attenuata*) kill of 1974 to 1983 was heavily biased towards mature animals (Barlow and Hohn, 1984). If the kill of eastern spinner dolphin was similar for all years, then the simple model used would have over-estimated relative population size. Removing proportionally more mature animals, whose reproductive value was highest, would have temporarily reduced the growth rate of the population and caused the population to decline for a longer period than predicted by the simple model.

In fact, an independent abundance index derived from data on sightings of dolphin schools from tuna vessels estimated that the population experienced a statistically significant decline from 1975, the first year the index was available, until 1982 (Buckland et al., 1992). This is different from the population trajectory I estimated here, which declined only until about 1977 (Fig. 5). Additionally, Buckland et al.'s (1992) trajectory indicated that the population level in 1988 was not substantially different from that of 1979, which conflicts with the model trajectories presented here with higher growth rates (Fig. 6), in which substantial growth occurs over 1979–1988. If Buckland et al.'s (1992) estimated population trajectory was an accurate assessment of the true population trend, then the results presented here suggest either 1) that the population growth rate was less than R_m=0.04; or 2) that kill was under-estimated during the 1980's for reasons discussed above; or 3) that a skewed age-structure led to a lagged response to the large decrease in kill during the 1970's; or 4) some combination of these possibilities.

Current status

Estimated kill from the fishery in recent years has been as high as 19,526, with an average kill of 13,900 from 1986 to 1990 (DeMaster et al., 1992), which represented a kill rate of 2.1% of the population estimate of 632,700. As indicated by Equation 3, the estimates of historical population size presented here, which are back-calculated from 1988, were based only on kill data through 1987. Estimated kill was 18,793 in 1988 (IATTC, 1989) and 15,245 in 1989 (Hall and Boyer, 1991), representing 3.0% and 2.4% of the abundance

[4]Gerrodette, T., and C. Perrin. 1991. Calibration of shipboard estimates of dolphin school size from aerial photographs. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Southwest Fish. Sci. Cent., P.O. Box 271, La Jolla, CA 92038. Admin. Rep. LJ-91-36, 24 p.

[5]Goodman, D. 1984. Consideration of age structure in back projection calculations for the northern offshore spotted dolphin population. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Southwest Fish. Sci. Cent., P.O. Box 271, La Jolla, CA 92038. Admin. Rep. LJ-84-26C, 25 p.

estimate, respectively. These recent kill estimates were the highest since 1976 (Table 2), and may have been high enough to prevent recent recovery of the population. The most recent estimates of the abundance index from tuna vessel sighting data indicated the population was declining from 1986 to 1991 (Anganuzzi et al., 1992[6]). However, the most recent kill information indicated a substantial reduction in kill to less than 1% of the population in both 1990 (5,378, Hall and Boyer, 1992) and 1991 (5,879, Hall and Lennert, in press), which resulted in an average kill per year for 1988–91 of 11,324, or 1.8% of the population estimate of 632,700. Therefore, the current status of the population is unlikely to be substantially different from what it was in 1988. Managing kill levels so that they do not exceed some fraction of the expected maximum net recruitment rate may be the most reasonable management strategy for promoting recovery of the population (DeMaster et al., 1992). The U.S. National Marine Fisheries Service has recently proposed listing the eastern spinner dolphin as depleted under the U.S. MMPA (Federal Register, 17 June 1992, 57FR27010). A separate proposal to list the eastern spinner dolphin as "threatened" under the Endangered Species Act was not warranted at this time, as the population is in no immediate danger of extinction (Federal Register, 19 October 1992, 57FR47620). Proposed international quotas on fisheries kill for each dolphin stock in the eastern tropical Pacific (IATTC, in press; MMC, 1993), if implemented, would ensure that mortality levels stayed low enough to allow recovery of the population to the OSP level.

Conclusions

Based on the best data available on abundance, kill, and population dynamics, the population size in 1988 of the eastern spinner dolphin was estimated to be below MNPL, within the range of 32% to 58% of pre-exploitation population size. Based on available life history data, the population size was estimated at 44% of pre-exploitation population size. Relative population size was estimated to be higher than Smith's (1983) estimate for 1979, but this difference was due mostly to the use of a new, better estimate of abundance, rather than to a recovery of the population between 1979 and 1988. Although there are uncertainties associated with this analysis, especially with the early kill data, the results indicated that the eastern spinner dolphin population was well below historical abundance levels in 1988. Most uncertainties appear to lead to over-estimates of relative population size, indicating the population may be at a lower level than indicated here. Calculation of confidence limits for relative population size showed that the precision of the estimates was sufficient to make a status determination except for higher values of R_m. However, higher growth rates ($R_m > 0.04$) were not supported by independent evidence available about the population trend since 1975. The results indicated that, as of 1988, the stock of eastern spinner dolphins was depleted as defined by the U.S. MMPA. The substantial fisheries kill that occurred after 1988 makes it unlikely that the population has experienced any significant recovery since then.

[6]Anganuzzi, A. A., S. T. Buckland, and K. L. Cattanach. 1992. Relative abundance of dolphins associated with tuna in the eastern Pacific Ocean: analysis of 1991 data. Paper SC/44/SM23 presented at the annual meeting of the Int. Whal. Comm.

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Spring distribution and abundance of ichthyoplankton in the tidal Delaware River

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Abstract.—Water quality in the tidal freshwaters of the Delaware River has improved substantially over the last decade. Areas near Philadelphia that were once anoxic now rarely experience dissolved oxygen concentrations lower than 3 ppm. To assess how fish spawning and nursery activity in the river has changed following these water quality improvements, ichthyoplankton were collected from April to June in 1987 and 1988 in the tidal freshwaters of the Delaware River and compared to data available from studies conducted during the mid-1970's. Eighteen taxa were collected in the present study; larval *Morone americana* (white perch), *Alosa sapidissima* (American shad), and *Alosa* spp. (river herring) were the most abundant. Total density of eggs and larvae was highest upstream of Philadelphia, where water quality has historically been highest. Ichthyoplankton density and taxonomic composition in the tributaries were similar to the most productive portions of the mainstem, except in the Schuylkill River. There, water quality is still poor, and ichthyoplankton density was an order of magnitude less than in the mainstem. In comparison to data from the previous decade, the present assemblage of the Delaware River was more diverse. Nine taxa, most notably *A. sapidissima*, were significantly more abundant in at least one portion of the river in the present study, though total ichthyoplankton density was no higher than prior to the water quality improvements. Five taxa, most notably *Cyprinus carpio* (carp), were significantly less abundant in the present assemblage.

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The tidal freshwater portion of the Delaware River was once among the most polluted estuaries in the United States. Water quality was so poor during the 1940's that gases released from the water reportedly tarnished metal and corroded engine parts of naval vessels moored near Philadelphia, PA (Albert, 1988). Conditions were poorest in the Philadelphia area, where much of the river became anoxic during warmer months. This so-called pollution block substantially reduced the upriver migration of anadromous fishes such as *A. sapidissima* and *Morone saxatilis* (striped bass) (Chittenden, 1971, 1974).

Installation of secondary treatment at municipal sewage treatment plants along the river has reduced organic loading by more than 75% since the early 1970's (Albert, 1988). As a result, dissolved oxygen concentration in the river has increased, and the magnitude and duration of the pollution block near Philadelphia has been reduced. In areas that were once anoxic, dissolved oxygen concentrations during the spring are now rarely lower than 5 ppm, or 3 ppm during the summer (Albert, 1988). Fishery resources appear to have responded to the improved water quality conditions; the size of the *A. sapidissima* spawning population has increased substantially (Maurice et al., 1987) and average catch of young-of-year *M. saxatilis* has increased by more than an order of magnitude[1].

Despite the size and importance of the Delaware estuary, little is known about which fish use the tidal freshwater region of the river as a spawning and nursery ground. The most recent regional ichthyoplankton survey was conducted in 1981, but covered 100 km of river with only 20 samples per week and only data for *M. saxatilis* were reported[2]. Numerous ichthyo-plankton collections were conducted in the mid-1970's as part of power plant or industrial-related impact studies, but these were individually limited in spatial extent and have never been integrated to provide a regional description. Most important, however, no regional study has been conducted since the dramatic improvements in water quality occurred. In this study we provide new data to describe present ichthyoplankton distribution and abundance in the tidal Delaware

[1] Weisberg, S. B., W. H. Burton, and H. A. Wilson. 1991. Delaware River striped bass studies: population estimate of the 1990 year class and an evaluation of the young-of-year index of abundance. Rep. to the Delaware Basin Fish and Wildl. Manage. Coop., Trenton, NJ.

[2] Himchak, P. J., J. Carlson, and R. Tilton. 1981. Spawning and recruitment of the striped bass, *Morone saxatilis*, in the Delaware River. Rep. available from New Jersey Dep. Environ. Protection, Trenton, NJ, Bureau of Marine Fisheries.

River and its tidal tributaries, integrate data from the many ichthyoplankton collections conducted during the 1970's, and compare these data to describe how spawning and nursery activity in the area has changed.

Methods

Ichthyoplankton samples were collected weekly from April to June in 1987 and 1988, following a stratified random design. Sampling in 1987 was conducted between Riverton, New Jersey (river kilometer [rkm] 174) and the fall line at Trenton, New Jersey (rkm 214) for nine weeks beginning on 22 April (Fig. 1). The sampling area was stratified into four 10-km regions. Each region was stratified further into three habitats: shoal (river depth of less than 8 m), channel bottom (within 1 m of the bottom in areas with an overall depth >8 m), and channel mid-water. Three randomly placed samples were collected from each habitat in each region during each week. Sampling in 1988 was conducted between Bristol, Pennsylvania (rkm 191), and Artificial Island, New Jersey (rkm 87), for eight weeks beginning on 18 April (Fig. 1). Allocation procedures in 1988 were the same as in 1987, except that the area was stratified into nine equally-sized regions, and in the channel bottom habitat, only two samples were collected.

The sampling of the river in 1987 also included tributary stations in Martin's Creek, Neshaminy Creek, Dredge Harbor, and Rancocas Creek. In 1988, tributary stations were established in the Schuylkill River, Raccoon Creek, Oldman's Creek, the Christina River, and at two stations in the Chesapeake Bay and Delaware River (C&D) Canal. Tributary stations were located approximately 1 km upstream of the confluence with the Delaware River, except for the second C&D Canal station, which was located near the Rt. 13 bridge, about 4 km from the river. One mid-water sample was collected from each tributary station each week, except for the C&D Canal stations, where both mid-water and bottom samples were taken weekly.

Samples from the bottom habitat were collected by using a 1-m diameter epibenthic ichthyoplankton sled; water column and shoal habitats were sampled using stepped oblique tows with a 0.5-m bongo net. Both types of gear were fitted with 505-μm mesh plankton nets. The volume of water that each net filtered was estimated with a General Oceanics digital flowmeter mounted at the mouth of the epibenthic sled and to one side of the bongo net. Tows were conducted against the current for five minutes at a speed of approximately 1.0 m/sec. The catch was preserved in 10% formalin, stained with rose bengal, and taken to the laboratory for analysis.

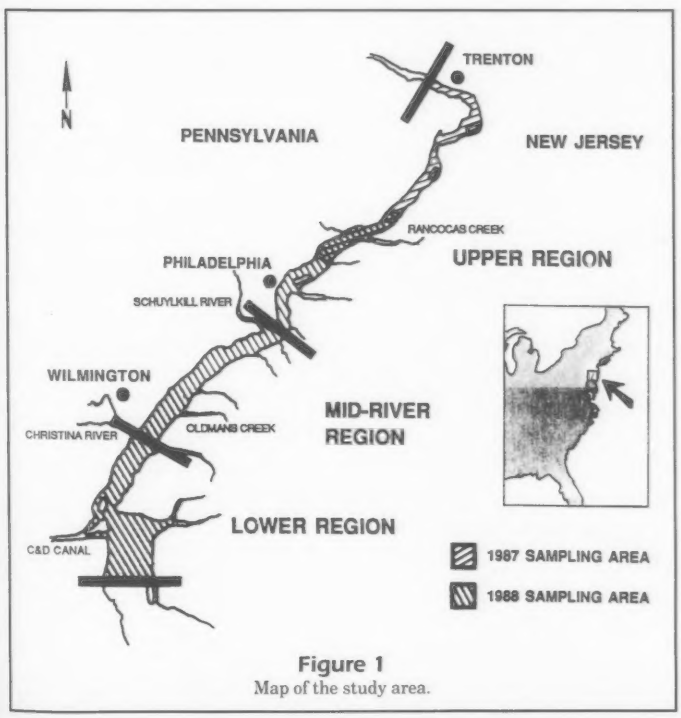

Figure 1
Map of the study area.

Physico/chemical data (temperature, dissolved oxygen, pH and conductivity) were collected weekly with a calibrated Hydrolab Surveyor II in each of the river regions, the C&D Canal, and at each of the tributary stations. Measurements in the river and in the C&D Canal were taken at surface, mid-depth, and bottom. In the tributaries, measurements were taken at surface and bottom. Water transparency was measured with a 20-cm Secchi disk.

In the laboratory, all eggs with diameters greater than 1.0 mm were removed from the collections and inspected to determine if they were *M. saxatilis* or *A. spidissima* based on the size of the perivitelline space relative to the diameter of the egg. Small eggs (less than or equal to 1.0 mm in diameter) consisted mostly of *M. americana*, *Alosa aestivalis* (blueback herring), or *Alosa pseudoharengus* (alewife), but were not differentiated quantitatively. *M. americana* yolk-sac larvae were dis-

tinguished from *M. saxatilis* larvae based on the smaller size of *M. americana* at this stage and on gut morphology, which in *M. americana* parallels the notochord for about six myomeres before turning downward. Post yolk-sac *M. americana* and *M. saxatilis* larvae less than 8 mm in length were separated by size and the formation of fin rays, (formation of fin rays occurs at about 5 mm and 8 mm, respectively). Post yolk-sac larvae of 8 mm and longer were identified by clearing and staining the specimens and examining the pterygiophore/neural spine interdigitation patterns (Olney et al., 1983). *A. sapidissima* larvae were separated from *Alosa* spp. by counting post-anal myomeres (Lippson and Moran, 1974[3]; Chambers et al., 1976).

For the purpose of data presentation, the Delaware River sampling effort was post-classified into lower river, mid-river, and upper river regions (Fig. 1). These regions corresponded to Artificial Island (rkm 87) to the Christina River (rkm 114), Christina River to the Schuylkill River (rkm 148), and Schuylkill River to Trenton, NJ (rkm 214), respectively. Density estimates were calculated for each region during each week as the simple average of all samples collected in that region. A Friedman's rank-sum test, blocked on week, was used to test for differences in density among regions (Conover, 1980).

Data from over 2,000 ichthyoplankton samples collected from the Delaware River during the 1970's were obtained from reports submitted as part of regulatory requirements or environmental impact studies (ANSP, 1974[4]; Anselmini, 1974[5]; Anselmini 1976[6]; Potter et al., 1974, a and b[7,8]; Harmon and Smith 1975[9]; PECO, 1977, a–e[10,11,12,13,14]; IA, 1979, a and b[15,16]; RMC, 1979[17]). While none of these studies individually provided coverage of the entire tidal river, collectively they provided excellent coverage. To compare present ichthyoplankton composition and abundance with that in the 1970's, these data were digitized and summarized.

To maximize comparability of data between periods, we extracted for analysis from the historical reports only those samples which were collected between mid-April and mid-June with a 500-μm mesh gear that was towed for at least five minutes and filtered at least 40 m^3. Where studies differed in taxonomic level of identification, data from all studies were restated to the higher taxonomic group. From these data, weekly average densities were estimated for each region and year. Historic abundances for each region were taken as the simple averages of weekly estimates. We included data for only years in which collections were taken in at least eight weeks between April and June to ensure that our comparison was not biased by data sets with limited temporal coverage. Comparisons of average abundance with the present study were accomplished separately for each region by using a Friedman's test blocked on week.

Results

Water quality

Water temperature during the 1987 and 1988 survey ranged from 11 to 26°C. Differences in temperature between regions were less than 1°C in both years.

[3] Lippson, A. J., and R. L. Moran. 1974. Manual for identification of early developmental stages of fishes of the Potomac River estuary. Available from Maryland Department of Natural Resources. Ref. No. PPSP–MP–13, 282 p.

[4] ANSP (Academy of Natural Sciences of Philadelphia). 1974. Ecological studies in New Jersey, Oldman's Creek, Raccoon Creek, Birch Creek, and the Delaware River 1972–1973. Prep. by ANSP, Philadelphia, Pennsylvania for the Shell Oil Company, Philadelphia, PA.

[5] Anselmini, L. D. 1974. An ecological study of the Delaware River in the vicinity of the Mercer Generating Station, Trenton, NJ. Prep. by Ichthyological Associates, Inc., Ithaca, NY, for Public Service Electric and Gas Co., Newark, NJ.

[6] Anselmini, L. D. 1976. An ecological study of the Delaware River in the vicinity of Newbold Island. Prep. by Ichthyological Associates, Inc., Ithaca, NY, for Public Service Electric and Gas Co., Newark, NJ.

[7] Potter, W. A., D. C. Smith, and P. L. Harmon. 1974a. An ecological study of the Delaware River in the vicinity of Chester Generating Station. Chester Progress Rep. 1. Prep. by Ichthyological Associates, Inc., Ithaca, NY, for the Philadelphia Electric Co., Philadelphia, PA, 94 p.

[8] Potter, W. A., D. C. Smith, and P. L. Harmon. 1974b. An ecological study of the Delaware River in the vicinity of Eddystone Generating Station. Eddystone Progress Rep. 3. Prep. by Ichthyological Associates, Inc., Ithaca, NY, for the Philadelphia Electric Co., Philadelphia, PA, 42 p.

[9] Harmon, P. L., and D. C. Smith. 1975. An ecological study of the Delaware River in the vicinity of Eddystone generating station. Eddystone Progress Rep. 4 to Philadelphia Electric Co., Philadelphia, PA.

[10] PECO (Philadelphia Electric Company). 1977a. Chester Generating Station, 316(b) Rep., Permit No. PA 0011614. Rep. to the EPA, Philadelphia, PA.

[11] PECO. 1977b. Richmond Generating Station, 316(b) Rep., Permit No. PA 0011649. Rep. to the EPA, Philadelphia, PA.

[12] PECO. 1977c. Southwark Generating Station, 316(b) Rep., Permit No. POA 00116-65. Rep. to the EPA, Philadelphia, PA.

[13] PECO. 1077d. Delaware Generating Station, 316(b) Rep., Permit No. PA 0011622. Rep. to the EPA, Philadelphia, PA.

[14] PECO. 1977e. Eddystone Generating Station, 316(b) Rep., Permit No. PA 00137-14. Rep. to the EPA, Philadelphia, PA.

[15] Ichthyological Associates (IA). 1979a. Effect of the cooling water intake structure, intrainment and impingement of fishes. Burlington Generating Station Demonstration for Section 316(b) of the Federal Water Pollution Control Act Amendments of 1972, PL 95-500. Prep. by IA, Ithaca, NY, for Public Service Electric and Gas Co., Newark, NJ.

[16] Ichthyological Associates (IA). 1979b. Effect of the cooling water intake structure, intrainment and impingement of fishes. Mercer Generation Station Demonstration for Section 316(b) of the Federal Water Pollution Control Act Amendments of 1972, PL 95-500. Prep. by IA, Ithaca, NY, for Public Service Electric and Gas Co., Newark, NJ.

[17] Radiation Management Corporation (RMC). 1979. An evaluation of the cooling water intake at the Edge Moor Power Station. Edge Moor Power Station section 316(b) Evaluation. Permit No. DE-000058. Prep. by RMC, Pottstown, PA, for Delmarva Power and Light Co., Wilmington, DE.

Temperature, dissolved oxygen, and salinity were not vertically stratified in the water column and did not differ substantially between tributaries and the nearest mainstem water quality sampling locations.

Average dissolved oxygen values decreased over the sampling period during each year of the study, ranging from greater than 10 mg/L during the first week to less than 8 mg/L during the last week. Dissolved oxygen differed by as much as 3 mg/L among regions, with the lowest values occurring downstream of Philadelphia. However, only a single measurement below 5 mg/L was recorded in 1988, and no measurements below 6 mg/L were recorded in 1987.

The river was consistently fresh upstream of Pea Patch Island (km 98); salinity averaged less than 2 ppt in the regions downstream of Pea Patch Island. The highest salinity (5 ppt) was observed in the C&D Canal during the second week of sampling in 1988. A storm and subsequent freshwater runoff during the sixth week of the 1988 sampling turned the entire mainstem sampling area fresh but had little effect on salinity in the C&D Canal, which never fell below 2 ppt.

Distribution of eggs and larvae

Eighteen taxa, comprising 15 families, were collected from the Delaware River during 1987 and 1988 (Table 1). Seventeen of these taxa also were found in the tributaries (Table 2); no taxon occurred only in the tributaries. Most taxa were not abundant. Only three,

Table 1

Average density (no./100 m^3) of ichthyoplankton in the lower, mid- and upper regions of the Delaware River during the present and previous (1972–1978) studies. Values in bold represent regional densities that were significantly ($P<0.05$) different between the present and previous studies.

	Present study			Previous studies		
	Lower river	Mid river	Upper river	Lower river	Mid river	Upper river
Percichthyidae						
Morone saxatilis (eggs)	0.2	0.2	<0.1	0.1	0.3	0
Morone saxatilis	0.1	**0.4**	0.1	0.3	0.1	0
Morone americana	0.5	**8.4**	48.3	1.1	0.4	27.2
Clupeidae						
Alosa sapidissima (eggs)	0.1	**0.1**	**0.4**	0	0	0.1
Alosa sapidissima	**0.8**	**15.0**	**49.0**	0	0	0
Other Alosa spp.	0.2	5.4	33.5	**4.1**	22.8	**339.7**
Brevoortia tyrannus	**0.1**	0.1	0	0	0	0
Dorosoma cepedianum	0	0	0	0	<0.1	0
Ictaluridae	0	0	0.1	0	0	0
Anguillidae						
Anguilla rostrata	<0.1	<0.1	<0.1	**0.8**	**0.4**	**0.2**
Percidae						
Perca flavescens	<0.1	0	**0.7**	0	0	<0.1
Etheostoma sp.	<0.1	<0.1	0.2	0	0	0.6
Cyprinidae	0.1	1.3	0.5	**1.0**	**6.8**	**10.5**
Soleidae						
Trinectes maculatus	**0.1**	**0.1**	<0.1	0	0	0
Centrarchidae						
Lepomis sp.	<0.1	<0.1	**0.1**	0	0	0
Pomoxis sp.	0	0	0	0.1	0	<0.1
Catostomidae						
Carpiodes cyprinus	<0.1	**0.4**	0.6	0	0	**3.4**
Castostomus sp.	0	0	0	0	0	**1.0**
Engraulidae						
Anchoa mitchilli	0.6	0.1	<0.1	0.2	0	0
Gasterosteidae	<0.1	<0.1	<0.1	0	0	0
Cyprinodontidae						
Fundulus sp.	0	0	<0.1	00.1	<0.1	
Sciaenidae						
Leiostomus xanthurus	**0.5**	**0.1**	<0.1	<0.1	0	0
Atherinidae						
Menidia sp.	0.2	<0.1	<0.1	0.4	0	0
Umbridae						
Umbra pygmaea	0	0	0	0	0	<0.1

Table 2
Average density (no./100 m³) of ichthyoplankton in the Delaware River tributaries among samples collected in 1987 and 1988.

	C&D Canal	Christina River	Oldman's Creek	Raccoon Creek	Schuylkill River	Dredge Harbor	Rancocas Creek	Neshaminy Creek	Martin's Creek
Percichthyidae									
Morone saxatilis (eggs)	5.7	0.2	0	0.2	0	0	0	0	0
Morone saxatilis	0	0	0.7	1.5	0	0	0	0	0
Morone americana	0.1	21.5	8.0	30.9	1.4	4.0	36.9	8.8	2.4
Clupeidae									
Alosa sapidissima (eggs)	<0.1	0	0	0	0	0	1.6	0	0
Alosa sapidissima	0	41.4	168.4	247.3	1.3	80.7	199.9	46.6	75.5
Other *Alosa* spp.	0	28.6	106.4	160.6	1.0	52.0	194.5	52.7	57.8
Anguillidae									
Anguilla rostrata	0	0	0	0	0	0	0	0.1	0
Percidae									
Perca flavescens	<0.1	1.0	0	0	0	0	1.3	0	0
Etheostoma sp.	<0.1	2.2	0.2	0.1	0	0	0.1	0.1	0
Cyprinidae	0	0.6	0.8	7.4	0.6	0	<0.1	2.1	0
Soleidae									
Trinectes maculatus	0.1	0	0	0	0	0	0	0	0
Centrarchidae									
Lepomis sp.	0	0	0	0.4	0	0.3	0.6	0.4	0.5
Catostomidae									
Carpiodes cyprinus	0	2.8	0.2	0.8	0	0.1	0.7	0.2	0
Engraulidae									
Anchoa mitchilli	0.1	0	0	0	0	0	0	0	0
Gasterosteidae	0.1	0	0	0	0	0	0	0	0
Cyprinodontidae									
Fundulus sp.	0	0	0	0	0	0	<0.1	0	0
Sciaenidae									
Leiostomus xanthurus	1.8	0	0	0	0	0	0	0	0
Atherinidae									
Menidia sp.	0.1	0	0	0	0	0	0	0	0

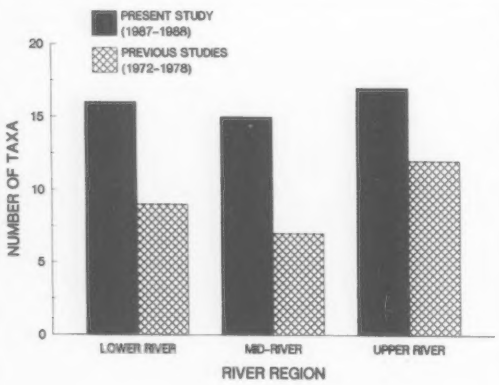

Figure 2
Number of ichthyoplankton taxa found in each of the three regions of the tidal Delaware River in the present (1987–1988) and in previous (1972–1978) studies.

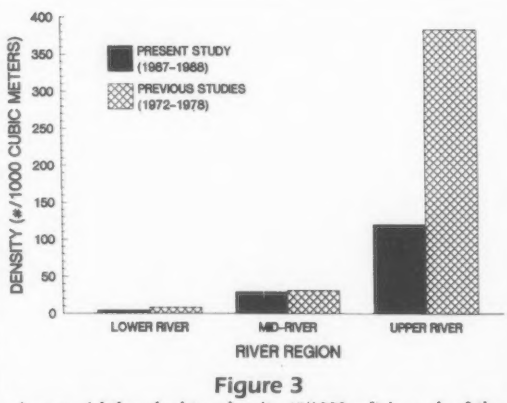

Figure 3
Average ichthyoplankton density (#/1000 m³) in each of the three regions of the tidal Delaware River in the present (1987–1988) and in previous (1972–1978) studies.

A. sapadissima, *M. americana*, and *Alosa* spp., occurred at average densities greater than 10/100 m³ in either the mainstem or the tributaries.

Numbers of taxa did not differ significantly among the three regions of the river (Fig. 2), but average ichthyoplankton density did ($P<0.001$) (Fig. 3). Density was greatest in the upper region (between the Schuylkill River and Trenton, NJ) where it averaged more than 120 larvae/100 m³ during the study. In contrast, density in the mid-river region (between Phila-

Table 3

Comparison of highest weekly ichthyoplankton density (no./100 m^3) found in the lower, mid, and upper river sampling regions between the 1987–1988 study and historical studies.

	Present study			Historical studies				
	Lower river	Mid river	Upper river	Lower river	Mid river		Upper river	
	(1988)	(1988)	(1987–1988)	(1978)	(1974)	(1977)	(1972)	(1973)
Percichthyidae								
Morone saxatilis (eggs)	0.6	0.6	0.1	0.3	0	2.9	0	0
Morone saxatilis	0.2	2.5	1.2	1.1	0	0.7	0	0
Morone americana	1.7	31.2	387.2	2.7	2.0	2.5	56.1	125.0
Clupeidae								
Alosa sapidissima (eggs)	0.2	0.4	1.4	0	0	0	1.7	0
Alosa sapidissima	3.5	52.0	219.6	0	0	0	0	0
Other *Alosa* spp.	0.9	26.8	151.9	14.5	202.5	51.3	3231.3	522.9
Brevoortia tyrannus	0.6	0.4	0	0	0	0	0	0
Dorosoma cepedianum	0	0	0	0	0	0.2	0	0
Ictaluridae	0	0	0.8	0	0	0	0	0
Anguilla rostrata	0.1	0.1	0.1	3.7	0.2	0.6	0.5	0
Percidae								
Perca flavescens	0.1	0	3.6	0	0	0	0.3	0.2
Etheostoma sp.	0	0.1	1.1	0	0	0	5.4	1.6
Cyprinidae	0.4	9.1	5.8	4.2	51.8	45.6	84.5	24.6
Soleidae								
Trinectes maculatus	0.4	0.2	0.1	0	0	0	0	0
Centrarchidae								
Lepomis sp.	0.1	0.3	0.3	0	0	0	0	0
Pomoxis sp.	0	0	0	0	0	0	0	0
Catostomidae								
Carpiodes cyprinus	0.3	3.3	2.1	0	0	0	26.4	0.7
Catostomus sp.	0	0	0	0	0	0	24.4	1.8
Engraulidae								
Anchoa mitchilli	1.8	1.1	0.4	0.7	0	0	0	0
Gasterosteidae	<0.1	0.2	<0.1	0	0	0	0	0
Cyprinodontidae								
Fundulus sp.	0	0	0.1	0	0.4	0	0	0
Sciaenidae								
Leiostomus xanthurus	1.8	0.2	<0.1	0	0	0	0	
Atherinidae								
Menidia sp.	0.5	0.1	<0.1	1.8	0	0	0	0
Umbridae								
Umbra pygmaea	0	0	0	0	0	0	0.3	0

delphia, PA and Wilmington, DE) was only about one-fourth of that in the upstream area. Average larval density in the lower region (downstream of Wilmington) was less than 4/100 m^3 and never exceeded 10/100 m^3 during any week of the study (Table 3).

Species composition was fairly consistent between the mid- and upper regions but differed substantially in the lower river region (Table 1). *M. americana, A. sapidissima*, and *Alosa* spp. were dominant in the upper river, occurring at densities of an order of magnitude greater than any other taxa. In the region furthest downstream, where salt intrusion begins to occur, species typical of the estuarine environment, such as *Leiostomus xanthurus* (spot) and *Anchoa mitchilli* (bay anchovies), were at least as abundant as *A. sapidissima* and *Alosa* spp. Freshwater species that occurred in the upper river, such as *Etheostoma* sp. (darters) and *Ictaluridae* (catfish), were not found in the more saline region.

With the exception of the Schuylkill River and the C&D Canal, density and taxonomic composition in the tributaries was similar to that in the most upstream region of the tidal mainstem (Table 2). The Schuylkill River is the largest tributary to the Delaware River and flows through a heavily industrialized portion of Philadelphia. Only four taxa were collected there, and they generally occurred at densities 10- to 100-fold lower than in any other tributary. No species occurred

there at a density greater than 10/100 m^3. The other anomalous tributary was the C&D Canal, which is not a true tributary, but a man-made, free-flowing canal constructed in the early 1900's to link the Delaware and Chesapeake estuaries for navigation. The C&D Canal contains virtually no freshwater drainage, and the species composition there was most similar to the lower region of the mainstem. No clupeid larvae and very few *M. americana* larvae were collected there, but the C&D Canal did contain densities of *M. saxatilis* eggs considerably higher ($P<0.01$) than the mainstem or other tributaries.

Potential bias in the recent survey could exist because sampling in the upper river region was primarily accomplished in 1987, whereas the downstream areas were sampled in 1988. To determine if this was a problem, we compared ichthyoplankton density and species composition in the area sampled in common between the two years. We found that the difference in total ichthyoplankton density between the two years was less than 10% (Table 4) and not significantly different. In addition, none of the dominant taxa had more than a two-fold difference in density between years. Temporal patterns for the dominant taxa were generally consistent across both years of the study (Fig. 4). Seasonal peaks in larval abundance for *Perca flavescens* (yellow perch) preceded that of the *Alosa* spp., followed by *M. americana* and *M. saxatilis*. *A. sapidissima* and *P. flavescens* larval abundance peaked about a week later in 1988 than in 1987. This difference may be attributable to inter-annual variability in spawning peaks; however, since sampling in 1988 was concentrated farther downriver, the difference in temporal patterns between years may partly reflect the time required for downstream transport of larvae from upstream spawning grounds.

Comparison with historical data

When we compared our data to the historic data, we found that average density of all eggs and larvae did not differ significantly in the two lower regions and that historical density was higher ($P<0.01$) in the most upstream region (Fig. 3). The larger difference among periods was in composition and dominance; the number of taxa caught in each region in the present study was almost double that in the historical data (Fig. 2), despite the greater number of sampling years encompassed by the previous studies. Whereas river herring accounted for more than half of the catch in every region during the 1970's, no taxon accounted for more than 40% of total catch in any region in the present study (Table 1).

Density of nine taxa increased significantly in at least one region since the previous decade (Table 1). Of these, the increase was most dramatic for *A. sapidissima*. In the earlier studies, *A. sapidissima* eggs were rarely found, and larvae were not encountered at all. In contrast, the density of shad larvae in the upper river region exceeded 100/100 m^3 during several weeks of our study (Table 3). Five taxa decreased significantly in number in at least one region, and cyprinids (minnows) and *Anguilla rostrata* (elvers) declined significantly in all three regions. Cyprinids were only about one-tenth as abundant in our study. Although we did not identify all of the cyprinids in our study, the cyprinids collected consisted primarily of juvenile *Notropis* spp., whereas most cyprinids in previous studies were identified as juvenile *C. carpio*.

Table 4
Average ichthyoplankton density (no./100m^3) in the area and weeks samples in common between 1987 and 1988.

	1987	1988
Percichthyidae		
Morone saxatilis (eggs)	0	<0.1
Morone saxatilis	0	0.1
Morone americana	23.7	38.4
Clupeidae		
Alosa sapidissima (eggs)	1.2	0.8
Alosa sapidissima	40.2	65.0
Other *Alosa* spp.	31.4	21.0
Anguillidae		
Anguilla rostrata	<0.1	0
Percidae		
Perca flavescens	1.7	0.5
Etheostoma sp.	0.2	1.0
Cyprinidae	0.1	0.4
Soleidae		
Trinectes maculatus	0	<0.1
Catostomidae		
Carpiodes cyprinus	1.1	0.2
Sciaenidae		
Leiostomus xanthurus	0	<0.1
Gasterosteidae	0	<0.1

Discussion

The dramatic decline of *A. sapadissima* productivity in the Delaware River since the last century (Chittenden, 1974) has been attributed to reductions in the amount of usable spawning habitat (Chittenden, 1976). Shad have historically used all of the Delaware River as spawning and nursery grounds, but spawning during most of this century was limited to areas 200-km upstream from the tidal freshwater areas we studied. Size of the spawning ground was reduced for two reasons (Chittenden, 1976). First, only the earliest arriving shad, which typically spawn farthest up-

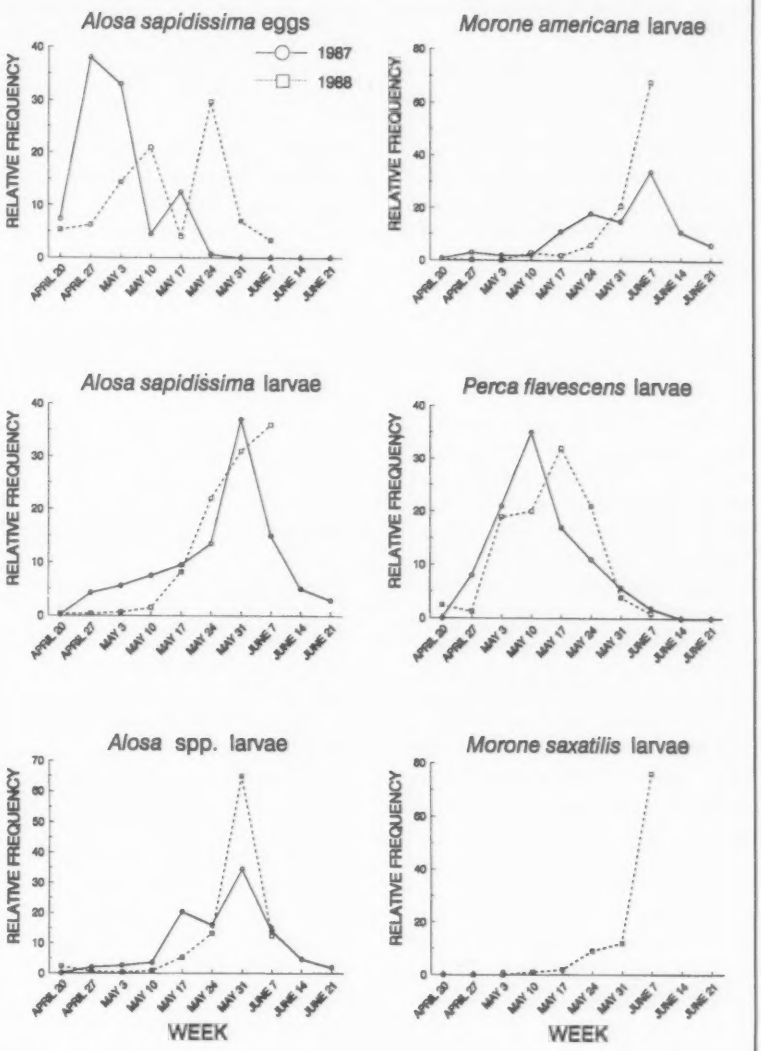

Figure 4

Temporal patterns of abundance for the dominant larval fish of the tidal Delaware River. Solid and dashed lines represent 1987 and 1988, respectively.

stream (Glebe and Leggett, 1981; Maurice et al., 1987), could get past Philadelphia before hypoxic conditions developed. Second, only fish migrating out of the river late in the season, that is fish spawned in the most upstream reaches, arrived in the Philadelphia area after the lethal dissolved oxygen conditions dissipated.

Our data suggest that the spatial extent of *A. sapidissima* spawning habitat in the Delaware River has expanded over the last decade, coincident with the water quality improvements near Philadelphia. While we found no evidence of spawning or nursery activity in tidal freshwater during the 1970's, prior to water quality improvements, by the late 1980's the density of larvae there was as high as in non-tidal upstream reaches. The suggestion of expanded spawning ground for shad is consistent with Maurice et al. (1987), who documented substantial increases in spawning activity during the last decade in the lower reaches of the

non-tidal river. Presumably, expansion of the spawning and nursery grounds is a first step in recovery of the stock.

There was little difference in *M. americana* abundance in the Delaware River between our study and the historical data (Tables 1 and 3), suggesting that the poor water quality may have had little impact on *M. americana* spawning and nursery activities in the Delaware River. We observed that most *M. americana* larvae were concentrated upstream of Philadelphia in an area where water quality was not as seriously impacted as below Philadelphia (Albert, 1988). Unlike *A. sapidissima*, which leave the estuary and must return through the Philadelphia region to spawn, *M. americana* remain in the estuary throughout their life cycle. Presumably, these life history characteristics have allowed *M. americana* to avoid effects of the poor water quality of the Philadelphia area more effectively than shad.

We found that larval *M. saxatilis* were primarily concentrated in the area downstream of Philadelphia. Since this area historically had the worst water quality, the *M. saxatilis* population should have benefitted from water quality improvements. The Delaware River beach seine monitoring survey, conducted annually by the State of New Jersey, showed a consistent increase in young-of-year *M. saxatilis* abundance throughout the 1980's[1]. However, we did not find the density of eggs to be higher in our study than in the 1970's, and the density of larvae was only slightly higher. We did find that present densities of *M. saxatilis* eggs and larvae in the Delaware River are considerably less than in the Hudson River[18] or the Chesapeake Bay (Setzler-Hamilton et al., 1981; Grant and Olney, 1991). This may be due, in part, to a year effect, since juvenile abundance in the Delaware River in 1988, as measured by the beach seine survey, was lower than in any other year since 1985. However, it is possible that higher juvenile abundance in the late 1980's resulted from better survival of larvae as water quality improved (Burton et al., 1992), rather than from increased egg production.

We found that the density of *M. saxatilis* eggs was considerably higher in the C&D Canal than in the mainstem Delaware River. This finding seems to support studies suggesting that the mechanism for maintenance of the Delaware River *M. saxatilis* population is transport of eggs spawned in the Chesapeake Bay through the C&D Canal (Chittenden, 1971; Johnson and Koo, 1975). Our data, however, are probably more consistent with that of Kernehan et al. (1981), who suggested that most of the eggs transported through the canal are not viable. In spite of finding considerably elevated densities of eggs in the C&D Canal, no larvae were found there, and we collected fewer than 10 larvae within 10 km of where the C&D Canal empties into the Delaware River estuary. Most of the *M. saxatilis* larvae we found in the Delaware River were collected between the Delaware Memorial (rkm 111) and Commodore Barry (rkm 132) bridges, more than 30 km upstream of the C&D Canal. This distance represents about three tidal excursion distances, and while upstream transport of eggs is possible in some estuarine systems (Norcross and Shaw, 1984), it is unlikely to occur in this portion of the Delaware because of the lack of a well-defined thermocline or pycnocline that would allow for two-layer circulation. Thus, it is more likely that most of the *M. saxatilis* larvae found in the Delaware River were actually spawned in the river.

[18] Lawler, Matusky, and Skelly Engineers (LMS). 1989. 1986 and 1987 year class report for the Hudson River estuary monitoring program. Prep. by LMS, Pearl River, NY, for Consolidated Edison Co., New York, NY.

Acknowledgments

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Effects of entanglement and escape from high-seas driftnets on rates of natural mortality of North Pacific albacore, *Thunnus alalunga*

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North Pacific albacore (*Thunnus alalunga*) are heavily fished and may have experienced recent reductions in recruitment (NOAA, 1991). U.S. troll fishery landings and catch per unit of effort (CPUE) have been decreasing since the 1960's and are currently at only about 50% of their peak levels (Kleiber and Perrin, 1991; NOAA, 1991). Landings and CPUE by the Japanese pole-and-line fishery have also been falling continuously since the late 1970's[1]. North Pacific albacore are additionally targeted by longline fleets, and considerable numbers are taken by high-seas large- and small-mesh driftnet fleets. The small-mesh fleets of Japan, Taiwan, and Korea target neon flying squid (*Ommastrephes bartramii*) and incidentally capture albacore. The large-mesh fleets of Japan and Taiwan target albacore, skipjack tuna (*Katsuwonus pelamis*), and various billfishes (*Istiophoridae* and *Xiphiidae*).

Foreign driftnet fleets and U.S. trollers taking albacore overlap geographically (Fig. 1). They also target a common stock even when fishing in widely separated areas because of the trans-Pacific migration of albacore (Otsu and Uchida, 1963). Albacore that encounter drift nets, and escape to survive long enough to be recaptured by another fishery can bear some external marks[2,3]. These marks provide direct evidence of interactions among fisheries. U.S. trollers operating in the North Pacific have reported an increased frequency of net-marked fish with the expansion of the Taiwanese, Korean, and Japanese high-seas driftnet fleets (NOAA, 1991;[2,3]).

Albacore that become entangled in drift nets face a number of possible fates (Fig. 2). The fraction that become entangled and subsequently drop out (alive or dead) is not known. If it is significant, the North Pacific albacore stock could be affected more seriously by high-seas drift nets than landings alone indicate. Our study, part of a larger effort to determine the impacts of drift nets on the North Pacific albacore stock, was undertaken to determine whether albacore that encounter drift nets, and subsequently escape alive, suffer higher than normal rates of natural mortality.

Our strategy was to look for differences in measures of fitness, and differences in short-term and long-term growth rates between net-marked (i.e., fish that have encountered drift nets and escaped) and unmarked albacore. We also looked for indications of bacterial infection and long-term stress in net-marked albacore. These parameters could evince, although not quantify, differences in rates of natural mortality. We had to use indirect measures because rates of natural mortality (or physiological changes) could not be directly observed in albacore encountering simulated drift nets in shoreside tanks. Unlike skipjack and yellowfin tuna (*Thunnus albacares*) (Brill, 1992), albacore have yet to be successfully maintained in captivity.

Selection of specific parameters was constrained by the necessity of collecting measurements and samples at sea aboard small commercial fishing vessels. We could acquire only measurements and samples that could be obtained under difficult conditions and that did not interfere with normal fishing operations. We were further limited to collecting samples that could be stored for later processing in the laboratory and that would not reduce the market value of the catch. Given these constraints, we chose the following parameters. Weight to length and maximum girth to length ratios were used as indicators of general fitness (Bolger and

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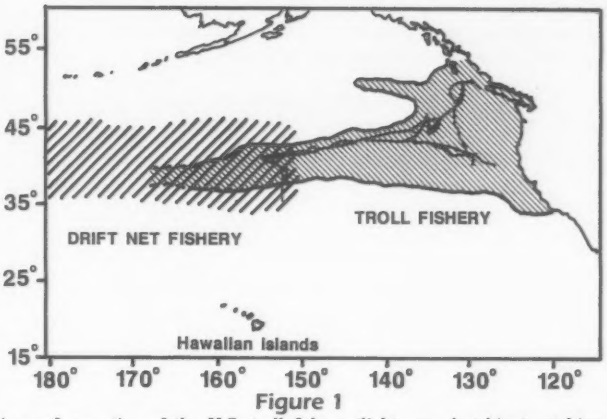

Figure 1

Area of operation of the U.S. troll fishery (light cross-hatching) catching albacore. Track lines of U.S. vessels carrying National Marine Fisheries Service observers in 1991 are shown by the solid line. Area of operation of foreign drift net fleets (heavy cross-hatching) extends west to approximately 140°W.

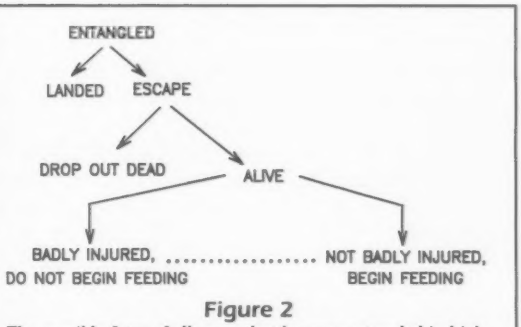

Figure 2

The possible fates of albacore that become entangled in high-seas drift nets. Note that the condition of fish which escape drift nets alive probably form a continuum from those that are so badly injured that they succumb quickly, to those that are only slightly injured and resume feeding immediately.

Connolly, 1989; Cone, 1989, 1990; Murphy et al., 1991). Relative otolith weight (i.e., the ratio of otolith weight to body weight) was used as an indicator of long-term growth rate (Boehlert, 1985; Pawson, 1990; Fletcher, 1991). (Because otolith weight is proportional to fish age rather than to body size, slower-growing fish have larger otoliths at a given body size because they have taken longer to reach that body size.) RNA:DNA ratio of white muscle was measured because it is an established indicator of short-term (days to weeks) growth rates (Bulow, 1970; Bulow et al., 1981; Ferguson and Danzmann, 1990; Mustafa et al., 1991). Relative leukocyte abundance (i.e., the ratio of leukocytes to red blood cells) was assessed as an indicator of bacterial infection and long term stress (Blaxhall, 1972; Anderson, 1990; Goede and Barton, 1990). Although muscle lipid content can be used as a measure of recent feeding history (Dotson, 1978), it was not used in this study. The lipid content of tuna muscle is dependent on sampling site (Dotson, 1978; Vileg et al., 1983; Boggs and Kitchell, 1991) and this factor could not be rigorously controlled.

Materials and methods

During 1990 and 1991, observers were placed aboard ten U.S. trollers by the Southwest Fisheries Science Center (NMFS, NOAA), in cooperation with the Western Fishboat Owners Association. Observers documented the number of net-marked albacore landed and collected data and samples for our study. Boats operated in an area of the North Pacific shown in Figure 1. Albacore were classified according to the severity of net damage with a scheme (Table 1) developed by Bartoo et al.[2]. Fork length, maximum girth (both to the nearest centimeter), and body weight (to the nearest kilogram) were measured. Dried blood smears, sagittae (otoliths), and white-muscle samples (frozen in sealed tubes in the boats' commercial fish freezers) were also obtained from a subsample of landed fish selected by the observers. Muscle samples (taken from the small amount of muscle remaining near the skull following decapitation) and otoliths could be obtained only on vessels that processed (i.e., "gilled and gutted") their catches. Complete sets of samples were not, therefore, obtained from every fish.

In the laboratory, otoliths were dried, cleaned, and weighed to the nearest 0.1 mg on a Sartorius 1207 MP2 electronic balance. RNA and DNA were extracted from the frozen muscle samples by standard procedures (Hutchison and Munro, 1961; Munro and Fleck, 1966; Wilder and Stanley, 1983). The only modifications were that the RNA was extracted with 1.0 N NaOH, and the DNA with 1.2 N perchloric acid, both at 37° C for 60 minutes. RNA, DNA, and protein concentrations were measured with a Beckman model 35 UV spectrophotometer using the dual wave length method (Tsanev and Markov, 1960) and standard extinction coefficients (Wilder and Stanley, 1983). Red cells and leukocytes were counted in Geimsa-stained blood smears with the aid of light microscopy (oil immersion lens) in a minimum of three microscope fields, or until at least 200 red cells were counted. Eosinophils could be easily differentiated from the remainder

Table 1

Net damage classification scheme (Bartoo et al.[2]) used to assess severity of injury to albacore caused by entanglement and escape from drift nets, and the number fish in each damage code category examined for our study.

Damage code	Damage description	Number of fish
0	No damage or scarring.	217
1	Minor damage along side(s) of fish, pattern of stripes due to minor scale loss where fish forced its way through or along the net.	39
2	Minor damage to head, chiefly forward of pectoral fins, brush-like pattern of scale loss.	5
3	Severe damage from bruising or scraping away of parts of the skin, primarily in area of greatest girth and mostly on dorsal surface.	10
4	Damage of any degree that was partially or completely healed.	60

of the leukocyte population and were excluded from the total leukocyte count. Although Alexander et al. (1980) described the distinguishing features of albacore blood cells, we were only able to differentiate eosinophils clearly from the remainder of the leukocytes. Leukocytes were, therefore, not further classified.

By using data from unmarked fish only, regressions of body weight on fork length, maximum girth on fork length, and otolith weight on body weight were fitted by a least squares procedure (Statgraf Statistical Software) to the exponential equation:

$$Y = a \cdot x^b.$$

Regression parameters a and b were estimated for each case.

Relative condition factor, relative girth, and relative otolith weight for individual fish were then calculated with the regression parameters:

$$K = W/(a \cdot X^b).$$

When K = relative condition factor, W = body weight and X = fork length; when K = relative girth, W = maximum girth and X = fork length; and when K = relative otolith weight, W = otolith weight and X = body weight.

Relative condition factor, relative girth, and relative otolith weight were calculated to permit direct comparison of groups containing individuals of different body sizes (Pollard, 1972; Brill et al., 1987).

The standard condition factor (C) was calculated separately for unmarked and net-marked fish:

$$C = W / FL^3$$

where W = body weight and FL = fork length.

Fish were grouped only as net-marked (damage codes 1-4, Table 1) and unmarked (damage code 0). Differences in mean values for unmarked and net-marked fish were evaluated by unpaired Student's t-tests with $P < 0.05$ taken as the maximum level for statistical significance.

Results and discussion

The only significant differences between net-marked and unmarked fish were in measures of absolute size (mean fork length, body weight, and maximum girth), which were greater for unmarked albacore (Table 2). These data suggest that smaller fish escape drift nets, survive, and are recaptured by the troll fleet more frequently than large fish. This appears to be confirmed by size-frequency distributions for the 217 net-marked and 114 unmarked fish included in this study (Fig. 3A). The two peaks in Figure 3A (at approximately 65 and 80 cm) represent 3- and 4-year-old fish, respectively[2] and imply that 4-year-old individuals predominate the unmarked group. In the net-marked group, 3- and 4-year-old fish are approximately equally represented. This result is, however, more likely due to either a sampling bias of the observers when choosing fish for inclusion in this study, or to time-area variations in fish size composition and the percentage of net-marked fish. The size-frequency distributions of

Table 2

Parameters measured to evaluate the effects of escapement from drift nets on the natural mortality of albacore *Thunnus alalunga*. *N* equals the number of observations.

	Unmarked fish		Net-marked fish	
Parameters	Mean ± SD	*N*	Mean ± SD	*N*
Body weight* (kg)	10.6 ± 2.4	182	8.6 ± 2.6	83
Fork length* (cm)	79.1 ± 7.4	215	73.5 ± 7.9	114
Maximum girth* (cm)	56.1 ± 5.7	214	51.8 ± 6.2	113
Condition factor (kg/cm^3)	0.0208 ± 0.0015	182	0.0210 ± 0.0017	83
Relative girth	1.00 ± 0.02	214	1.00 ± 0.03	113
Relative condition factor	1.00 ± 0.07	182	1.00 ± 0.07	83
RNA to DNA ratio	6.99 ± 0.97	34	6.90 ± 1.49	36
Relative otolith weight	1.00 ± 0.09	175	0.99 ± 0.11	47
Leukocytes/200 red cells	7.26 ± 5.30	15	6.44 ± 3.59	35

*$P \leq 0.05$.

a much larger number of albacore (12,085 unmarked and 381 net-marked individuals[4]; and from which the fish used in this study are a subsample, show that the frequency distributions of 3- and 4-year-old net-marked and unmarked fish are more nearly identical (Fig. 3B); 3-year-old fish were found to be only slightly dominant in both groups.

Our objective in calculating condition factor, relative girth, and relative condition factor (also referred to as relative weight) was to determine if there was any indication of differences in fitness between unmarked and net-marked fish. No differences were found (Table 2). The use and misuse of condition factor and relative condition factor have received much recent attention (Bolger and Connolly, 1989; Cone, 1989; Murphy et al., 1991). The use of such indices in groups of fishes with different mean lengths and size-frequency distributions may not be justified because disparities may be due to differences in body size, rather than to physiological fitness (Cone, 1989, 1990). Because indices of fitness were not different for unmarked and net-marked fish, we considered the question of suitability moot.

No differences were found either in measures of short-term (RNA:DNA ratio) or long-term (relative otolith weight) growth rates (Table 2). The RNA:DNA ratio can be affected by the age of the fish (Haines, 1973); however, it was not corrected for body size (i.e., age) because we found no correlation between it and body weight.

The relative leukocyte counts also did not differ between unmarked and net-marked albacore (Table 2). We assumed that net-marked albacore could show either elevated or reduced leukocyte counts. Acute bacterial infection has been shown to increase leukocyte counts in fish (Wedemeyer et al., 1990). Conversely, reduced leukocyte counts can be caused by elevated circulating corticosteroid levels (an acute stress response) or by chronic bacterial infection causing leukocytolysis (Shreck, 1981; Wedemeyer et al., 1983). The lack of difference between unmarked and net-marked albacore suggests that none of the processes that can affect relative leukocyte counts was occurring in the latter.

We were hampered in interpreting our results because we could not determine precisely how long the fish were at liberty following escapement from drift nets. Based on observations of skipjack and yellowfin tuna held in shoreside tanks at the Kewalo Research Facility (described in Brill, 1992), skin damage resulting from capture generally heals completely within weeks in feeding fish (R. W. Brill, unpubl. observations). Fish with damage codes 1–3 (Table 1), therefore, probably encountered drift nets within one month of recapture. It was impossible to estimate how long fish with damage code 4 had been at liberty or how long net damage remains visible, although Bartoo et al.[2] speculated that fish with damage code 4 may have been at liberty more than a year since encountering a

[4]N. Bartoo, D. Holts, C. Brown, and L. Halko. La Jolla Laboratory, Southwest Fish. Sci. Cent., unpubl. data.

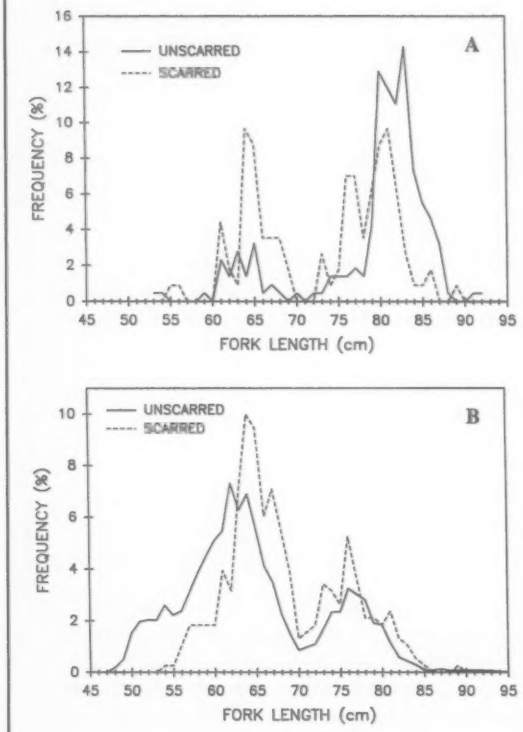

Figure 3

Size-frequency distribution of the unmarked and net-marked albacore sampled for our study (**A**) (215 unmarked and 114 net-marked fish) and much larger data set (**B**) (12085 unmarked and 318 net-marked fish) from which the fish used in our study are a subset (N. Bartoo, D. Holts, C. Brown, and L. Halko, SWFSC, La Jolla Laboratory, unpubl. data).

drift net. It is unknown if this is sufficient time for differences in relative otolith weight to become measurable. Boggs and Kitchell (1991) showed that significant weight loss occurs in tunas within one week of starvation. Therefore, it was likely that net-marked fish were at liberty long enough for measures of physiological fitness to become apparent, assuming net-marked albacore had not been feeding prior to recapture. The rate at which relative leukocyte abundance changes in albacore following stress or infection is not known, but in coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*O. mykiss*) leukocyte abundance changes within 96 hours of imposition of stressful conditions (McLeay and Gordon, 1977). Leukocyte abundance should be a good measure of differences in the health of net-marked and unmarked albacore, but the large standard deviation (Table 2) may limit its usefulness in this species.

Failure to detect differences (Table 2) between unmarked and net-marked albacore suggests that the fish escaping from drift nets, and living long enough to become vulnerable to recapture by U.S. trollers, do not suffer increased natural mortality. Most of the fish however, recaptured by trollers are found to be only minimally damaged or to have healed scars (Table 1), which may explain the lack of differences between net-marked and unmarked fish. As shown in Figure 2, there is most likely a spectrum of damage caused during encounters with drift nets. We do not know if the distribution of damage severity in the population of albacore vulnerable to the troll fishery is the same as that of all the albacore that escape drift nets alive. Also, the number of albacore falling out dead, or escaping so badly injured that they succumb before becoming vulnerable to recapture by troll vessels, may well be significant but remains to be determined.

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Weight change of pink shrimp, *Pandalus jordani*, after commercial harvest and handling

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The northeast Pacific trawl fishery for pink shrimp, *Pandalus jordani*, is unusual in that it is primarily managed by an aggregate size limit. Vessels are required to land catches with a minimum average size of 353 shrimp per kg (160 shrimp per lb). The regulation is intended to limit fishing mortality on age-one shrimp, and also to maintain the economic value of the catch (PFMC, 1981). The decision to retain shrimp from a particular tow is made by fishermen at sea; however, the regulation is enforced when the shrimp are landed in port, up to five days later. In general, a knowledge of how commercial fishing practices can influence the average weight of target species is important for effective management by an aggregate size limit (Kirkley and DuPaul, 1989). Accordingly, the objective of this study was to quantify changes in the average weight of pink shrimp from initial retention to shoreside delivery, using normal commercial handling and icing procedures.

Methods

To assess the weight change of shrimp from commercial handling practices, we compared the weight of shrimp samples taken directly from the trawl nets of commercial vessels at sea to the weight of the same samples during the subsequent shoreside delivery. Four to eight replicate samples of approximately 500g were collected from each tow and weighed to the nearest 0.1 g with a magnetically dampened triple beam balance. The accuracy of the scale was checked by using a known weight prior to each trip and again prior to weighing the samples at the dock. The scale used had also been previously tested for accuracy at sea with blind weighings and had been shown to produce measurements with a coefficient of variation of less than 0.5% under a variety of sea conditions[1]. Each sample was placed in a 5-mm mesh bag, labeled and passed to a crew member. Shrimp were taken directly from the vessel's hopper and were not washed prior to weighing and bagging. The crew was instructed to distribute the sample bags throughout the other shrimp from the same tow. After placement of the samples, the crew shoveled and raked flake ice into the layers of shrimp, according to that vessel's normal procedures.

At the completion of the fishing trip, the bags were retrieved during unloading and inspected for punctures. Punctured bags were discarded. Each intact sample was opened, placed in a tub with a 7-mm mesh bottom to drain for one minute, and weighed again with the same scale. An estimate of the percentage of ice in the gross load was obtained from processing plant records.

Weight change was expressed as a percentage of the original sample weight. The average percent weight change for all samples combined, for each trip and for each day of fishing, was compared to a hypothetical weight loss of zero percent by using a one sample *t*-test. To facilitate comparisons of weight change between trips or between individual days of fishing, 95% confidence intervals were constructed for each estimate of average weight loss. Scatter plots of percent weight change for individual samples versus elapsed time were constructed for each trip and for all trips combined. Each graph was tested for a significant slope with simple linear regression.

Results and discussion

Seven sampling trips were completed aboard commercial shrimp vessels between April and August 1988, lasting from 1 to 5 days. We recovered 70% of the mesh bags intact, resulting in weight change estimates for 713 samples from 132 tows. Elapsed time from catch to unloading ranged from 4 to 121 hours.

Percent weight change for the combined samples showed an approximately normal distribution centered on a mean weight loss of 2.2%, with a 99% confidence inter-

[1] M. Saelens and R. W. Hannah. 1988. Oregon. Dep. of Fish and Wildlife, Newport, OR 97365. Unpub. data.

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Fishery Bulletin, U.S. 91:804–807 (1993).

Table 1

Mean percent weight change, standard error of the mean and number of days on ice, between capture and unloading, for 500-g samples of pink shrimp, *Pandalus jordani* (number of samples is in parentheses). Asterisks denote results significantly different from zero percent weight change.

Trip	Days on ice					All days combined
	1	2	3	4	5	
1	−1.2 ± 0.9 (15)					−1.2 ± 0.009 (15)
2	0.4 ± 0.4 (11)	−0.3 ± 0.5 (14)	−1.8 ± 0.8 (9)	−4.1 ± 1.3 (3)		−0.7 + 0.4 (37)
3	−5.9[a] ± 0.8*** (14)	−0.4 ± 0.5 (25)	−1.8 ± 0.5** (17)	1.3 ± 0.6* (35)	−3.1 ± 0.9** (33)	−1.5 ± 0.4*** (124)
4	−2.1 ± 0.3*** (20)	−1.3 ± 0.3*** (28)	0.7 ± 0.5 (42)	0.9 ± 0.4* (30)		−0.2 + 0.2 (120)
5	−2.6 ± 0.3*** (23)	−5.4 ± 0.9*** (23)	−2.5 ± 0.5*** (24)	−2.2 ± 0.8* (19)	−4.6 ± 0.7*** (12)	−3.4 ± 0.3*** (101)
6	−4.7 ± 0.3*** (34)	−4.1 ± 0.4*** (43)	−5.2 ± 0.5*** (46)	−4.4 ± 0.5*** (32)		−4.6 ± 0.2*** (155)
7	−2.7 ± 0.2*** (24)	−2.0 ± 0.3*** (36)	−1.3 ± 0.4** (35)	−2.5 ± 0.3*** (45)	−0.4 ± 0.5 (21)	−1.9 ± 0.2*** (161)
All trips						−2.2 + 0.1*** (713)

[a] No ice used on this day of fishing. *P<0.05; **P<0.01; ***P<0.001.

Table 2

Summary of sampling trips, including trip length (d), total net landed catch (kg) and the estimated percentage of ice in the gross load (from processing plant records).

Trip	Trip length (d)	Total catch (kg)	Estimated percentage ice
1	1	600	35
2	4	7,900	16
3	5	15,000	17
4	4	11,900	23
5	5	5,200	32
6	4	25,200	7
7	5	14,000	11

val of 1.9% to 2.5% (Table 1). Four of the seven trips showed a statistically significant average weight loss (P<0.05), whereas three did not. None of the trips showed a gain in average weight of the shrimp samples, despite ice deductions as high as 35% of the gross load (Table 2).

The average weight change for samples from individual days and trips showed a large degree of variability. Several of the regressions of percent weight change versus elapsed time were statistically significant (P<0.05); however, the sign of the slope coefficient was inconsistent (Table 3). No consistent patterns were observed between average weight loss and trip length, total catch, or the percentage of ice in the load (Tables 1 and 2). The average weight loss observed in samples which had been on ice for only a short period of time (Table 1 and Figure 1), in combination with the significant positive slope of several of the regressions, suggests that commercially handled, iced pink shrimp generally lose some weight very shortly after capture, and, in many instances, slowly gain weight for some time afterwards. However, the one regression with a positive intercept and significant negative slope (Table 3, trip 2), in combination with the variability of average weight change (Table 1), demonstrate that weight change of shrimp under actual commercial handling and icing conditions is quite variable. The lack of a truly consistent pattern of weight change versus elapsed time suggests that Figure 1 may portray shrimp weight change under actual commercial fishing conditions as well as anything else presented.

Table 3

Summary of linear regression analysis of percent weight change on elapsed time for each trip and all trips combined. Model is Percent Weight Change = $a + b$ (Elapsed Time). NS = not significant.

Trip	Intercept	Coefficient	$P>F$
1	–0.207	0.013	0.0002
2	0.023	–0.001	0.0001
3	—	—	NS
4	–0.024	0.00041	0.0001
5	—	—	NS
6	—	—	NS
7	–0.027	0.00014	0.0238
All trips	–0.029	0.00013	0.00027

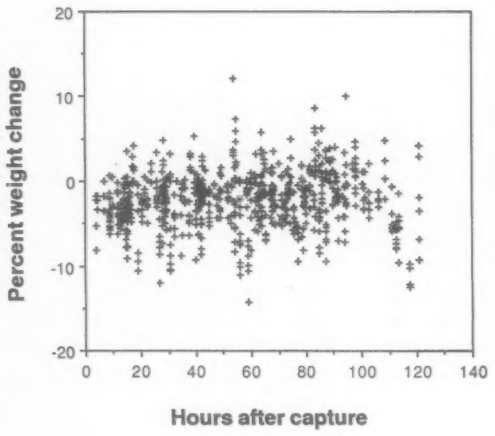

Figure 1

Percent weight change versus elapsed time from capture to unloading for samples of trawl caught pink shrimp (N = 713)

Interestingly, there were daily average weight change values which exhibited a significant positive change (Table 1), probably owing to absorption of meltwater from the ice. Samples from two trips showed large deviations from the average weight change which were readily explainable. On trip 3, the vessel ran out of ice on the final day of fishing, and, on trip 6, some spoilage was found throughout the load suggesting inadequate icing. In both cases the samples showed high weight loss values, 5.9% and 4.6% respectively.

These data suggest that there are small but fairly consistent losses in average weight of pink shrimp between commercial capture and unloading, at least when the shrimp are adequately iced. The results of this study appear to conflict with the findings of Bullard and Collins (1978), that iced *Pandalus borealis* gained 11% in weight in the first 1.5 days and maintained this gain for 8.5 days. However, the different methods used readily explain the different findings of the two studies. Bullard and Collins (1978) worked with shrimp which had been held at –1.7°C for two hours and then rinsed and drained for 30 minutes to produce stable weights prior to icing. The shrimp employed in this study were very fresh, sometimes jumping off the scales. If the average weight loss observed in this study occurs very rapidly, perhaps owing to loss of fluids just after the shrimp have died, or from the initial crush of the catch in the hold, such a loss could not have been detected in the Bullard and Collins (1978) experiment. It is not clear what percentage of ice was used by Bullard and Collins (1978), but they refer to it as "an excess of ice." Given the weight gain observed in some of the samples in this study, and the modest percentages of ice (Table 2), the results of the two studies are not inconsistent. Perhaps, however, the differences do suggest the importance of studying the weight change of shrimp under both laboratory and actual commercial fishing conditions.

An average weight loss of 2.2% from capture to unloading is sufficiently small as to be insignificant from a management standpoint. The minimum aggregate size limit of 353 shrimp per kg (160 shrimp per lb), which is intended to provide some protection from harvest for age-one shrimp, is based on average size-at-age data. Interannual and geographic variability in shrimp growth easily exceeds 2.2% (Hannah and Jones, 1991). The findings of this study are significant however, principally in that they demonstrate that the change in weight of pink shrimp that should be expected, when using normal icing procedures, is fairly small. Accordingly, fishermen can readily comply with the minimum size regulation.

Acknowledgment

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Occurrence and seasonal variations of spiny lobsters, *Panulirus argus* (Latreille), on the shelf outside Bahía de la Ascensión, México

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The spiny lobster, *Panulirus argus*, is one of the most valuable fishery resources on the Mexican Caribbean coast (Secretaría de Pesca, 1987). Lobsters are caught with traps and tangle nets, as well as by SCUBA diving, only on the shelf around Isla Mujeres and Isla Contoy (Fig. 1A), at significantly greater depths than elsewhere on the coast (Seijo et al. 1991). From Puerto Morelos to Xcalak, where the continental shelf is extremely narrow, lobsters are captured mainly by skin diving in the shallow coral reef and lagoon areas (Lozano-Alvarez et al. 1991a).

In Bahía de la Ascensión (Fig. 1B), the fishery for *P. argus* is based on the use of artificial shelters, called "casitas." Casitas are deployed on the bottom and the lobsters sheltering beneath them are caught by skin-diving fishermen. Both casitas and the fishing method are thoroughly described elsewhere (Lozano-Alvarez et al. 1989, 1991a; Briones et al. in press). Casitas are installed only within the bay, but some fishermen also skin dive for lobsters in the shallow coral reefs adjacent to the bay. No lobster fishing is conducted on the coastal shelf outside the bay deeper than 15 m. Only the tails are used. Tails are graded according to weight, packed in 10-pound (4.65 kg) boxes, and frozen. Fishing regulations include a minimum size limit of 13.5 cm tail length (≈74 mm carapace length, CL), a prohibition on the catching of egg-bearing females, and a closed season from 1 March to 30 June.

In 1985–87, results from a tagging program showed that the spiny lobster population within the bay comprised mostly juveniles and subadults (Lozano-Alvarez et al. 1991a). Growth of lobsters in the bay was fast, and they moved from the bay area towards the coral reefs as they grew. No evidence of reproductive activity in bay lobsters was found (Lozano-Alvarez et al. 1991a). Size of onset of sexual maturity is ≈80 mm CL, but females do not become fully mature until approximately 90 mm CL (Fonseca, 1990). This raised the hypothesis of the existence of the reproductive segment of the population on the shelf outside the coral reef, at greater, currently unfished depths. Lozano-Alvarez et al. (1991a) stressed the need to test this hypothesis for future management plans.

In this paper, we present evidence of the occurrence of adult reproductive *P. argus* on the continental shelf outside Bahía de la Ascensión, and discuss seasonal variations in abundance and some biological aspects of this segment of the population, as well as its potential importance as a refugium in space. The fisheries aspects of the study were published elsewhere (Lozano-Alvarez and Negrete-Soto, 1991).

Methods

Bahía de la Ascensión (Fig. 1B) is a large (≈740 km²) and shallow (<6 m deep) bay, bordered by mangroves and grass swamps. A substantial part of the bottom of the bay is covered with seagrass (mainly *Thalassia testudinum*) and dense aggregations of red and green algae (Briones et al., in press). A coral reef tract exists along its mouth, protecting the inner waters of the bay from wave surge. The bay is a nursery area for *P. argus* (Lozano-Alvarez et al. 1991a).

Fishing operations for spiny lobsters were carried out on the continental shelf outside the coral reef tract (Fig. 1B) during two different seasons, summer (11 July–10 September 1989, six fishings), and winter (14 November 1989–27 February 1990, five fishings). Standard lobster traps of the kind used by fishermen at Isla Mujeres were set. The traps were rectangular, measuring 121 × 91 × 40 cm. The frame, built with 12-mm diameter rod, was covered with plastic coated galvanized wire mesh (5 × 2.5 cm), and an entrance was left on one of the small sides. Traps were baited with cow hide.

A maximum of 39 traps was installed in any fishing operation, in lines parallel to the coral reef. The continental shelf in front of the bay is narrow, not exceeding 4 km from the coast. Complex reefs exist in this area at depths of <10 m and from 30 to 40 m (Jordán-Dahlgren et al., in press). After 40 to 60 m the slope becomes very steep, rapidly reaching depths in excess of 400 m. Echosounding was used to

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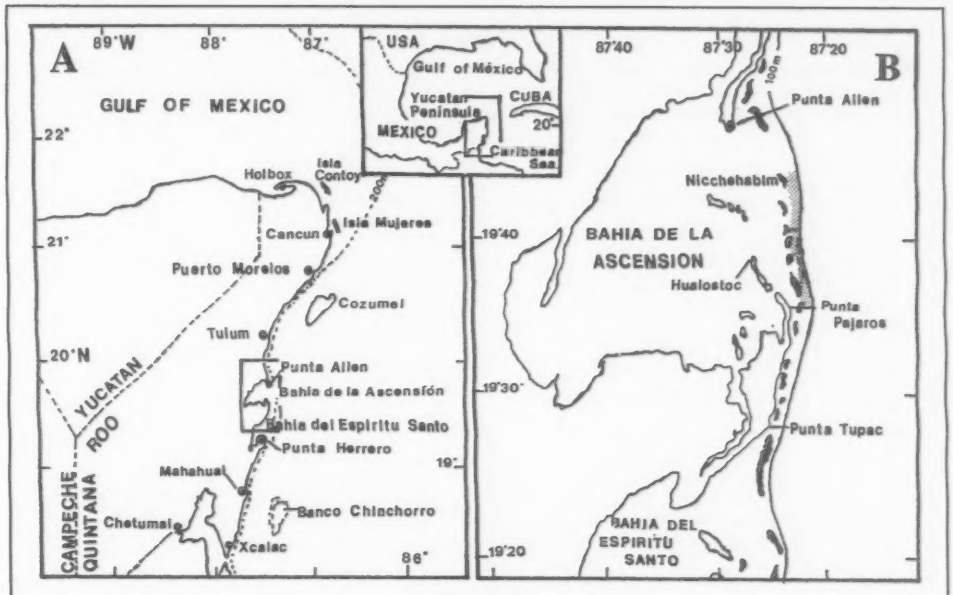

Figure 1

(A) The coast of Quintana Roo, showing the main fishing localities for *Panulirus argus*. (B) Bahía de la Ascensión. Black areas denote coral reef tracts; shaded areas show the sector of the shelf where exploratory fishings with traps were carried out. Casitas for lobster fishing were used coastward from the coral reef.

locate suitable bottoms for installing traps to minimize trap loss. In certain areas where the deep reefs are well developed (e.g., offshore from Punta Allen, Fig. 1B), no suitable bottoms were found. Thus, fishing operations were constrained to a range of depths between 15 and 30 m, from Nicchehabim Reef to Punta Pájaros (Fig. 1B).

Because soak time does not affect catch rate of the traps (Lozano-Alvarez et al., 1991b, Lozano-Alvarez and Negrete-Soto, 1991), we considered the number of lobsters per trap-lift as a gross index of abundance. Carapace length (CL) of lobsters was measured (±0.1 mm) from between the rostral horns to the posterior dorsal edge of the cephalotorax. Carapace state of lobsters was recorded following an arbitrary scale from 1 (newly molted and completely clean) to 4 (heavily fouled), as a relative indicator of the time elapsed since the last molt (Kanciruk and Herrnkind, 1976). Females were considered non-reproductive (NR) when eggs were absent, the carapace was clean, and no traces of spermatophores were found on the sternum. Reproductive females comprised those that presented a new spermatophore attached to the sternum (SP); external eggs attached to the pleopods (EE); or empty egg capsules remaining on the pleopods (EC).

To compare the size distribution and general features of the lobsters found on the shelf outside the bay with those from the bay, two different groups of data were obtained from the bay area. Throughout the fishing season, July 1989–February 1990, monthly data from a number of boxes of lobster tails, categorized by tail weight (TW, g) class, were obtained from a local processing plant. This plant receives all the catch from the bay area. The data were transformed to CL by applying the following equation: Log TW (g)= 2.550 log CL (mm) –2.69298 (Lozano-Alvarez et al., 1989). However, because the size structure of the catch is constrained by the minimum size limit and includes lobsters caught on the fore-reef down to a depth of 15 m, and the catch data gives no information on sex or reproductive activity, we also used data from Lozano-Alvarez et al. (1991a) taken during a tagging operation carried out in May–June 1986, which comprised lobsters found solely beneath casitas throughout the bay, as a less biased estimate of size structure, sex ratio, and carapace state of lobsters in the bay.

Student's *t*-test (Zar, 1984) was used to compare log-transformed data on seasonal abundance of lobsters (number of lobsters per trap-lift), mean sizes between males and females, and mean sizes between shelf and bay lobsters. Sex-ratios were compared with a χ^2 test. Mean sizes of summer and winter lobster catches taken on the shelf outside the bay were compared with a

Table 1

Number of traps lifted, number of lobsters (*Panulirus argus*) caught, and number of lobsters obtained per trap-lift during exploratory fishings with traps (summer 1989 and winter 1989–1990), on the continental shelf outside Bahía de la Ascensión, México.

Date of trap-lifting	No. of traps lifted	No. of lobsters	Lobsters per trap-lift
Summer			
Jul 19, 1989	36	0	0
Jul 24	21	16	0.76
Aug 10	18	23	1.28
Aug 16	13	5	0.38
Aug 22	34	14	0.41
Sep 05	34	9	0.26
Total summer	156	67	0.43
Winter			
Nov 23, 1989	39	106	2.72
Nov 26	30	29	0.97
Dec 05	39	204	5.23
Dec 08	13	21	1.62
Feb 27, 1990	39	35	0.90
Total winter	160	395	2.47

Table 2

Catch composition of *Panulirus argus* caught on the shelf outside Bahía de la Ascensión during the exploratory fishings with traps (summer 1989 and winter 1989–1990), and of those obtained from beneath casitas in the bay during May–June 1986 (M=males, F=females, CL=carapace length in mm).

	Offshore		
	Summer	Winter	Bay[a]
N	67	395	1402
Male: Female	1.31:1	1.04:1	1.04:1
Mean CL, F.	89.3	78.9	64.4
CL range, F.	55.0–156.9	52.4–117.2	22.0–100.3
Mean CL, M.	111.0	88.7	65.9
CL range, M.	76.5–162.1	56.0–134.0	29.8–113.1

[a] Data from Lozano-Alvarez et al. (1991a).

Table 3

Percentage of *Panulirus argus* in each of the four carapace states, caught on the shelf outside Bahía de la Ascensión during the exploratory fishings with traps (summer 1989 and winter 1989–1990), and of those obtained from beneath casitas in May–June 1986. Carapace states 1–4 represent a range from newly molted and completely clean to heavily fouled.

	Offshore		
Carapace state	Summer (*N*=67)	Winter (*N*=395)	Bay (*N*=1402)
1	35.82	74.43	55.99
2	37.31	18.99	37.44
3	17.91	6.07	6.13
4	8.95	0.50	0.42

non-parametric Mann-Whitney *U*-test with normal approximation (Zar, 1984). The level of significance considered throughout this paper is $P = 0.05$.

Results

Size structure and catch composition of lobsters on the shelf outside the bay

A total of 462 lobsters, 239 (52%) males and 223 (48%) females, were caught during fishing operations outside the bay. The observed sex ratio was not significantly different from the expected value (χ^2=0.554, df=1). Males were significantly larger than females (t=25.06, df=460, P<0.001). Mean CL for males was 92.2 mm (range: 56.0–162.1 mm) and for females was 80.4 mm (range: 52.4–156.9 mm).

Of the total catch obtained outside the bay, only 67 individuals (29 females and 38 males) were caught during the summer, while 395 (194 females and 201 males) were captured in the winter. Abundance of lobsters on the shelf outside the bay, expressed as number of lobsters per trap-lift, was significantly higher

Table 4

Reproductive state by size class of female *Panulirus argus* caught on the shelf outside Bahía de la Ascensión during exploratory fishings with traps (summer 1989 and winter 1989–1990). NR=non-reproductive; SP=new spermatophore on sternum; EE=external eggs on pleopods; EC= empty egg capsules on pleopods. Reproductive females comprise states SP, EE and EC.

Size class (CL, mm)	Summer				Winter			
	NR	SP	EE	EC	NR	SP	EE	EC
<80	8	0	0	0	112	0	0	0
80-85	3	1	1	0	21	0	1	6
85-90	1	1	0	1	27	1	0	4
90-95	5	0	0	1	1	2	0	5
95-100	0	0	0	1	2	0	0	3
100-105	0	0	0	2	1	0	0	2
105-110	0	0	0	1	1	2	0	0
110-115	1	0	1	0	0	0	0	1
>115	0	1	0	0	1	0	0	1
Total	18	3	2	6	166	5	1	22

during the winter than during the summer (t=4.21, df=9, P< 0.01) (Table 1). No significant differences in sex ratio were found between the two seasons (χ^2=1.332, df=1). The mean CL of the whole catch (Z=8.25, df=460, P<0.001), and of both males (Z=5.99, df=237, P< 0.001) and females (Z=4.15, df=222, P<0.001), was significantly greater in summer than in winter (Table 2).

Newly molted lobsters (carapace state 1) were more abundant in winter (74.4%) than in summer (35.8%), whereas lobsters with old, heavily fouled exoskeletons were more evident in summer (8.95%) than in winter (0.5%) (Table 3). During the summer, 11 (38%) of the 29 females were reproductive, while only 28 (14%) of the 194 females caught in winter were in this condition (Table 4). In both seasons, reproductive females measured over 80 mm CL, and most were in stage EC (Table 4). Only 2 females in summer and 1 in winter were actually carrying eggs (EE).

Size structure and catch composition of lobsters from casitas within the bay

The 1,402 lobsters caught beneath the casitas during May–June 1986 comprised 713 (51%) males and 688 (49%) females. No significant difference from unity was found in sex ratio. Mean CL of males (65.9 mm) was significantly larger (t=2.50, df= 1000, P<0.01) than that of females (64.4 mm) (Table 2, data from Lozano-Alvarez et al., 1991a). Most of the lobsters within the bay were in carapace states 1 and 2, i.e., recently molted (Table 3). Only five of the 688 females from within the bay were reproductive (stage EC), but these were among the largest in size (87.0–100.3 mm CL), and were found in sites close to the reef (Lozano-Alvarez et al. 1991a).

Comparison of size distribution between the shelf and bay segments of the population

Figure 2 shows the size distribution of a) the sample taken from casitas within the bay (modified from Lozano-Alvarez et al. 1991, a and b) the total sample taken on the shelf outside the bay. Both males and females were significantly larger on the shelf than in the bay (t=25.88, df=948, P<0.001 for males, and t=18.47, df=917, P<0.001 for females), but females composed a small proportion of shelf lobsters ≥90.0 mm CL (Fig. 2B).

Size structure of lobsters in the commercial catch from the bay and adjacent shallow reefs

Table 5 shows the monthly size distribution of the catch taken by the fishermen. Peak catches occurred in the 69.3–76.3 mm CL size class (which includes the minimum legal size) throughout the fishing season, except in July, where the peak was located in the 76.3–82.4 mm CL class. Lobsters ≥102.2 mm CL probably represented the catch from the adjacent shallow reefs, and represented a small proportion of the catch during most of the fishing season.

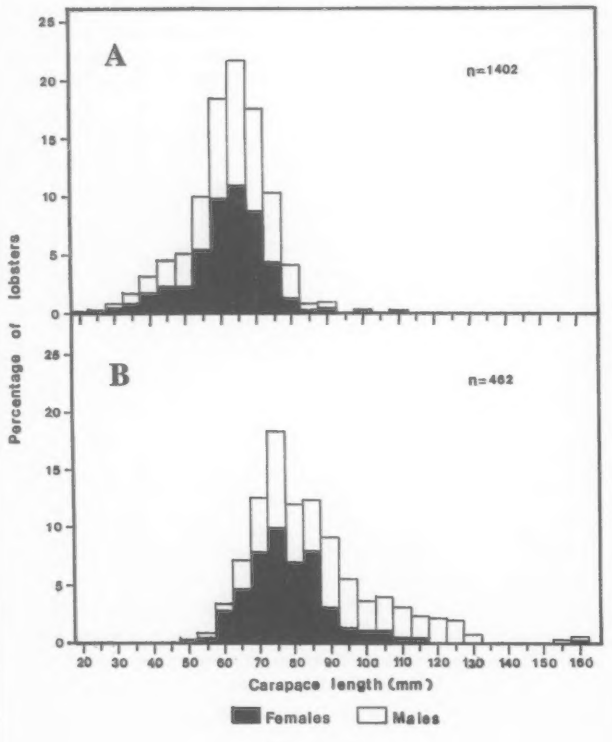

Figure 2

Size-frequency distribution of male and female *Panulirus argus* caught (**A**) beneath casitas within Bahía de la Ascensión (modified from Lozano et al. 1991a), and (**B**) in traps on the continental shelf outside the bay.

Discussion

The occurrence of a reproductive segment of the population of *Panulirus argus* on the shelf outside Bahía de la Ascensión has been documented in this study. The large sizes of lobsters and the evidence of mating and spawning activity on the continental shelf indicate that reproduction takes place in this deeper, unfished habitat, as opposed to the smaller sized, non-reproductive segment of the population within the bay.

Munro (1974) suggested that in coralline areas it is unlikely that the amount of shelter would be a limiting factor for spiny lobsters, but that this might be important in shelf areas with sparse coral cover. The deep and complex reef formations on the shelf outside Bahía de la Ascensión (Jordán-Dahlgren et al., in press) likely offer abundant natural shelters for *P. argus*. Lobsters are attracted to traps for refuge rather than for food (Heatwole et al., 1988), and traps apparently fail to adequately sample resident individuals (Herrnkind, 1980). Thus, the poor catches obtained during our summer fishings probably reflected the occupation of the available natural shelters by resident adult lobsters. The resident nature of these lobsters seems to be further supported by the large proportion of reproductive females and the high incidence of lobsters with fouled carapaces. The existence of resi-

Table 5

Monthly size-frequency distribution of the commercial catch (July 1989–March 1990) of *Panulirus argus* in Bahia de la Ascension, obtained by converting tail weight (TW, g) to carapace length (CL, mm)*.

Commercial category (TW, g)	Size range (CL,mm)	Number of lobsters							
		Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
60–100	56.7–69.3	1,484	848	636	1,274	636	424	0	371
100–128	69.3–76.3	17,720	6,680	5,240	4,640	3,200	2,680	2,401	1,600
128–156	76.3–82.4	18,464	5,408	3,680	3,456	2,144	1,760	2,240	1,184
156–185	82.4–88.1	12,717	3,672	2,592	2,241	1,431	1,134	540	621
185–213	88.1–93.2	6,785	2,461	1,656	1,472	897	690	690	391
213–241	93.2–97.8	3,480	1,460	960	880	560	420	402	240
241–270	97.8–102.2	1,692	900	612	468	306	342	180	162
270–298	102.2–106.3	944	432	352	288	160	240	0	128
298–340	106.3–111.9	810	480	285	255	150	180	0	105
340–397	111.9–118.9	572	338	195	195	117	156	130	91
≥397	≥118.9	486	348	191	110	81	171	190	91
Total		65,154	23,027	16,399	15,279	9,682	8,197	6,773	4,984

* Log TW = 2.55 Log CL –2.69298 (Lozano-Alvarez et al., 1989).

dent populations of *P. argus* has been suggested in the Bahamas (Kanciruk and Herrnkind, 1978; Herrnkind and Lipcius, 1989) and the Dry Tortugas (Davis, 1974).

In contrast, the greater abundance of lobsters on the shelf during the winter, in addition to their smaller mean size and recently molted carapaces, indicates a recent recruitment of subadult lobsters to the deep habitat. The recruits likely originated in the bay, from where they exited in pulses, as suggested by the large number of lobsters caught in two fishings (Table 1). These two fishings were conducted immediately after the passage of two severe cold fronts. Offshore movements of *P. argus* associated with cold fronts have been documented in other parts of the Caribbean and Florida (Herrnkind, 1980). Moreover, catches increase notably in winter in the deep fishery off Isla Mujeres, whereas catches from more shallow areas, such as Bahía de la Ascensión and Banco Chinchorro (Fig. 1A) decline at that time of year (Lozano-Alvarez, 1992). However, lobsters moving along the coast (Davis, 1979; Gregory and Labisky, 1986; Fonteles-Filho and Corréa-Ivo, 1980; Herrnkind, 1980) could also account for the greater abundance found on the shelf. Some lobsters tagged in Bahía de la Ascensión were recovered at locations several kilometers to the north and south of the bay (Lozano-Alvarez et al. 1991a). The extent of these movements along the offshore depth contours remains unknown, and warrants further investigation.

In contrast to the sex-ratio in our shelf samples, females outnumbered males in offshore *P. argus* populations in Florida (Davis, 1974; Lyons et al., 1981; Hunt et al., 1991[1]). Although in the Mexican Caribbean the reproductive season spans from March to November, with significant peaks in August–September (Fuentes-Castellanos, 1988), fully mature females, particularly those bearing eggs, were very scarce in our shelf samples. However, considerable evidence exists suggesting that traps fail to sample gravid females in several species of spiny lobsters (Herrnkind, 1980). We believe that gravid females were underestimated in our shelf samples owing to the availability of natural shelter.

The distribution of lobsters in and outside Bahía de la Ascensión seems to conform to the known size-related distribution of *P. argus*, that is to say juveniles inhabite shallow habitats (lagoons, bays) and adults dwelling on deeper reef habitats. However, some large adult lobsters were found in the bay and shallow adjacent reefs (Fig. 2A, Table 5). This fact suggests that some adult lobsters return to inshore habitats after their ontogenetic migration to deeper habitats. Inshore movements of large *P. argus* have been documented in Antigua and Barbuda (Peacock, 1974), Brazil (Fonteles-Filho and Corréa-Ivo, 1980) and Florida (Gregory and Labisky, 1986; Hunt et al., 1991). Hunt et al. (1991) suggested that these inshore movements are controlled by changes in behavior associated with reproduction.

The *P. argus* population in Bahía de la Ascensión and adjacent offshore areas is highly dynamic. Fishing pressure on juveniles in the bay and near shallow reefs is heavy (Lozano-Alvarez, 1992), but the closed season is strictly observed, and no lobster fishing is conducted offshore. Developing an alternative trap fishery off Bahía de la Ascensión would be impractical and costly, because of the narrowness of the shelf, the complex morphology of the bottom, the strong currents encountered in the zone, and the small and variable CPUE of legal-sized lobsters obtained (Lozano-Alvarez and Negrete-Soto, 1991). Consequently, the fishery will continue to be focused on the small-sized lobsters in the shallow areas.

Despite heavy fishing in the bay, we found evidence of a winter offshore migration. Lozano-Alvarez (1992) estimated a high emigration rate from the bay throughout the year, so other offshore recruitment pulses may occur at other times of the year (e.g., the closed season). Because of their large sizes, the resident females on the shelf outside the bay have high indices of egg productivity (Fonseca, 1990), and spawn more than once in the reproductive season (Lipcius, 1986). Thus, the unfished deep habitat outside the bay may bear importance as a refugium in space (Campbell, 1989; Caddy, 1990) for reproductive lobsters.

Other deep, currently unfished lobster areas were found offshore from Puerto Morelos (Lozano-Alvarez et al., 1991b) and probably exist elsewhere along the coast of Quintana Roo, particularly on the narrow shelf from Puerto Morelos to Xcalak (see Fig. 1A), and in many other areas of the Caribbean as well (e.g. Cuba, González et al., 1990). Their existence might serve to mitigate, through the production of larvae, the pressure exerted on more heavily fished stocks. We propose that the deep habitats on the shelf outside Bahía de la Ascensión should be left undisturbed as a nucleus of protected spawning stock, regardless of the unknown final destination of the larvae. We similarly encourage the protection of other deep, currently unfished areas throughout the Caribbean, as a means to preserve groups of reproductive lobsters that contribute larvae to the regional pool.

[1] Hunt, J. H., T. R. Matthews, D. Forcucci, B. S. Hedin, and R. D. Bertelsen. 1991. Management implications of trends in the population dynamics of the Caribbean spiny lobster, *Panulirus argus*, at Looe Key National Marine Sanctuary. Final Rep., NOAA Office of Ocean and Coastal Resource Management, Sanctuary Programs Div., Contract 50-DGNC-6-00093, 81 p.

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Growth and maturation of winter flounder, *Pleuronectes americanus*, in Massachusetts

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Winter flounder, *Pleuronectes americanus*, is an important commercial and recreational species along the Atlantic coast. Previous studies have shown that the winter flounder stock is composed of several substocks that may consist of estuarine specific populations (Saila, 1961; Poole, 1966a; Pierce and Howe, 1977). Coastal stocks of winter flounder are managed by individual states; area specific growth and maturity information is incorporated into spawning stock biomass-per-recruit models (ASMFC, 1992).

For management purposes, two stocks of winter flounder inhabit Massachusetts waters; one stock north of Cape Cod and the other stock south and east of Cape Cod (Lux et al., 1970; Howe and Coates, 1975; Pierce and Howe, 1977). For these stock units, growth parameters and sex ratios were reported from 1964 to 1968 coastal tag returns (Howe and Coates, 1975), but no studies using aged scale or otolith samples had been undertaken. Growth rates estimated from tagging may not be equivalent to those based on age-length data (Francis, 1988). The two methods often provide different results; faster growth rates are generally estimated from tag returns.

The primary objective of our study was to determine growth rates of juvenile and adult winter flounder based on aged scale samples. Our second objective was to determine average maturity schedules for the two winter flounder stock units, as maturation is an important life history parameter for spawning stock biomass-per-recruit analyses.

Materials and methods

Winter flounder were sampled during the 1983–91 Massachusetts Division of Marine Fisheries spring (May) bottom trawl surveys. State waters (0–3 mi) were surveyed with an otter trawl equipped with a fine mesh (13-mm stretch) codend liner that retained small fish. An average of 95 stations per year were surveyed in a stratified random manner, at a sampling intensity of 1 station per 19 square nautical miles. Additional details of trawl survey methodology were provided by Howe (1989). Captured winter flounder were measured to total length (±0.5 cm), sampled for scales and otoliths, and assigned sex and maturity stage based on examination of the gonads, by using established macroscopic criteria (Burnett et al., 1989). Because samples were collected at the end of the winter flounder spawning season, determination of sex and maturity stage was relatively straightforward. Scale samples, taken from the caudal peduncle region, were used for age determination. Scales were impressed in acetate and aged as described for Georges Bank winter flounder (Fields, 1988).

Von Bertalanffy growth curves were fitted to length-at-age data (1983–91 pooled) for males and females of each stock unit, by using mean lengths observed for ages 2–8. Data from age-1 fish were not included, as preliminary analysis suggested that the smaller fish of this age group were either not available or not effectively captured by the sampling gear. Mean lengths at age for older (>8 yrs) fish were not used in fitting growth models, because sample sizes were small. Statistical comparison of the results from this study and those of Howe and Coates (1975) was not attempted, as growth parameters derived from tagging and age-length information may not be equivalent (Francis, 1988).

Stock-specific maturity schedules based on aged fish were calculated for both males and females. For each sex within each stock unit, a logistic function was fitted to the proportion of mature winter flounder, ages 2–5, by using nonlinear regression. Lengths at 50% maturity (L_{50}) were the inflection point on these curves. Similarly, ages at 50% maturity (A_{50}) were determined from logistic curves fitted to observed maturity at age data.

Results

A total of 3,035 winter flounder (731 males and 986 females south and east of Cape Cod and 509 male and 809 female winter flounder north of

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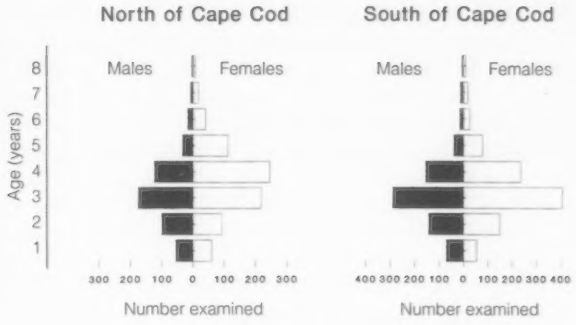

Figure 1
Sample sizes of male and female winter flounder taken from each stock area, 1983–91.

Cape Cod) were successfully aged and assigned maturity stages. Ages ranged from 1 to 16 years in the southern stock, and 1 to 15 years in the northern stock. For both stocks, females predominated at ages 3 and older (Fig. 1).

Growth rates of winter flounder differed for each gender and stock unit. Females grew faster than males, and obtained larger mean asymptotic lengths (Fig. 2). For each gender, winter flounder from the southern stock were generally larger at age than those from the northern stock, at ages 3–8. Mean lengths at age observed from our samples were similar to, but slightly smaller than, those determined from 1964–68 tag return data (Table 1).

For both stocks and sexes, maturation generally began at age 3 and was nearly complete at age 5. Maturation of age-3 and age-4 fish was quite variable and was somewhat sensitive to size at age (Fig. 3). Owing to their somewhat faster growth rate, winter flounder matured at younger ages south of Cape Cod. For females, A_{50}'s (and corresponding L_{50}'s) were 3.0 years (28.3 cm) south of Cape Cod and 3.3 years (28.7 cm) north of Cape Cod. For males, A_{50}'s (and corresponding L_{50}'s) were 3.1 years (28 cm) south of Cape Cod and 3.3 years (27.2 cm) north of Cape Cod.

Discussion

Adult winter flounder south and east of Cape Cod were larger at age than adults from other coastal populations. For example, age-5 female winter flounder in

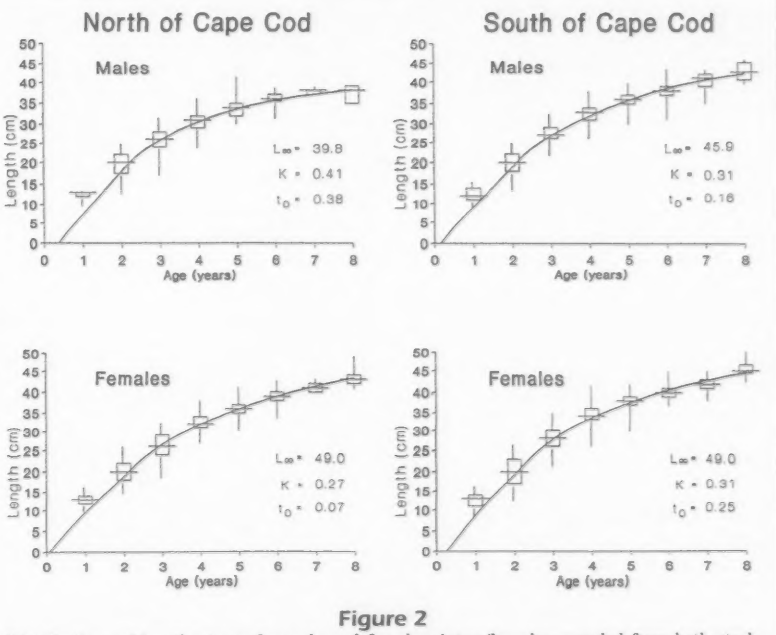

Figure 2
Distributions of length at age for male and female winter flounder sampled from both stock units. Von Bertalanffy growth curves are fitted through mean lengths, ages 2-8. Box plots of the median, quartiles, and range of lengths at age are shown.

Table 1

Calculated total length (cm) at age (yr) for female winter flounder in Massachusetts from 1983–91 scale samples (present study) and from 1964–68 tag returns (Howe and Coates, 1975).

	North of Cape Cod		South of Cape Cod	
Age	1964–68	1983–91	1964–68	1983–91
2	—	19.9	—	20.5
3	29.0	26.8	29.4	28.1
4	34.1	32.0	35.0	33.6
5	37.6	36.1	39.0	37.7
6	40.1	39.1	41.8	40.7
7	41.8	41.4	43.8	42.9
8	43.0	43.2	45.2	44.6

Massachusetts averaged 38 cm south and east of Cape Cod, versus 36 cm north of Cape Cod, 21 cm in Long Pond, Newfoundland (Kennedy and Steele, 1971), and 35 cm in Narragansett Bay, Rhode Island (Berry et al., 1965). On Long Island, New York, Poole (1966a) found that growth rates of winter flounder increased from south to north; age-5 females ranged from an average of 31 cm in Great South Bay to 36 cm in Peconic Bay. Only the offshore population on Georges Bank is known to have faster growth rates than winter flounder in Massachusetts waters south and east of Cape Cod (Lux, 1973; Howe and Coates, 1975). The area south of Cape Cod is the center of the species geographical range (ASMFC, 1992) and may provide better environmental conditions for growth of winter flounder than other coastal areas.

For both stocks of winter flounder in Massachusetts, growth was apparently not affected by changes in exploitation or biomass. Biomass of these stocks declined over 50% from 1983 to 1990 owing to high fishing mortality (Howe et al., 1990). However, no trends in mean lengths at age were observed for the 1983–91 period, and growth rates were similar to those observed by Howe and Coates (1975) for the 1964–68 period (Table 1), when winter flounder were more abundant and exploitation rates were lower[1]. Under high levels of exploitation, faster growing fish are differen-

[1] Foster, K. L. 1987. Status of winter flounder (*Pseudopleuronectes americanus*) stocks in the Gulf of Maine, southern New England, and middle Atlantic areas. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Northeast Fish. Sci. Cent. Ref. Doc. 87–06.

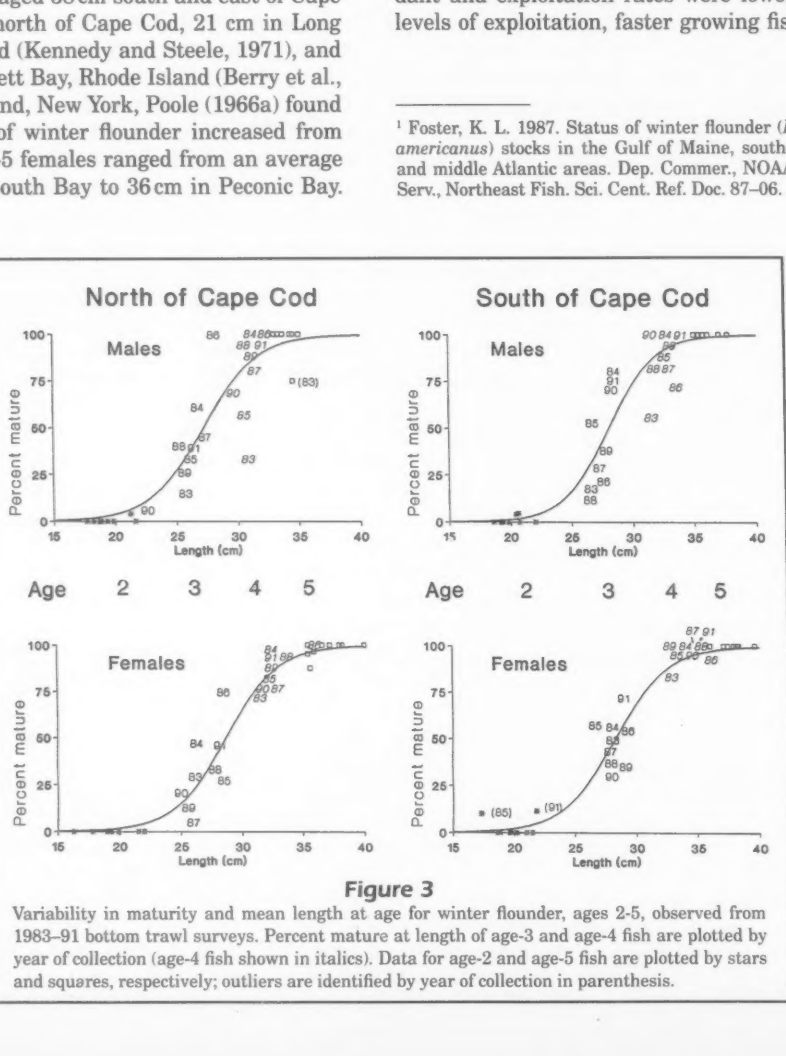

Figure 3

Variability in maturity and mean length at age for winter flounder, ages 2-5, observed from 1983–91 bottom trawl surveys. Percent mature at length of age-3 and age-4 fish are plotted by year of collection (age-4 fish shown in italics). Data for age-2 and age-5 fish are plotted by stars and squares, respectively; outliers are identified by year of collection in parenthesis.

tially removed from populations (Ricker, 1975), and thus we were unable to separate the effects of cropping faster growing fish from potential density dependent effects.

Winter flounder in Massachusetts waters mature at larger sizes than winter flounder from other coastal populations. For winter flounder in Massachusetts, only 50% of the females were mature at 28 to 29 cm; L_{50}'s of females in Newfoundland were 25 cm (Kennedy and Steele, 1971) and 27 cm in New Jersey (Danilla, 1978). Maturity ogives derived from our age-specific mean length at maturity data were similar to those reported from pooled length at maturity observations for Massachusetts winter flounder during the period 1985–89 (O'Brien et al., 1993). They found lengths at 50% maturity of females to be 27.6 cm south of Cape Cod and 29.7 cm north of Cape Cod. Because populations of long lived, late maturing fish are more susceptible to overexploitation than are populations with early maturity and shorter life spans (Pitcher and Hart, 1982), each substock of winter flounder may require different management strategies (including size limits) for optimal harvest levels.

Sex ratios of winter flounder favored females at older ages (Fig. 1), indicating a higher natural mortality rate for males. An increasing proportion of females by size was observed for winter flounder in Rhode Island, but the differences in sex ratios were thought to be related to the faster growth of females (Berry et al., 1965). Female winter flounder on Georges Bank live longer than males (Lux, 1973). A number of mature male winter flounder in Conception Bay, Newfoundland, failed to produce gametes, and there was a trend towards an increasing proportion of nonreproductive individuals with advancing age, leading to the possibility that males enter senescence at younger ages than do females, and thus have a higher natural mortality rate (Burton and Idler, 1984). A higher natural mortality rate for males has been found for other flounders, including European plaice, *Pleuronectes platessa*, (Bidder, 1925) and summer flounder, *Paralichthys dentatus*, (Poole, 1966b), and it may be a general pattern that male flounders have shorter life spans than females.

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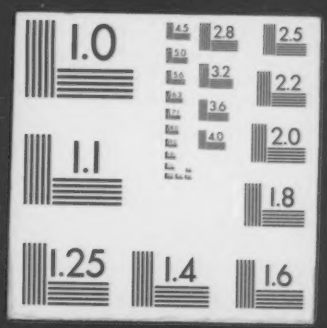
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